

## REVIEW

# A systematic meta-analysis based review on black soldier fly (*Hermetia illucens*) as a novel protein source for salmonids

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**Funding information**

This work was part of a PhD program funded by the SureAqua Nordic Center of Excellence (Grant no. 82342) and the Research Council of Norway (RCN), BioTek 2021/Havbruk Biofeed (Grant no. 229003).

**Abstract**

Black soldier fly (*Hermetia illucens*) has gained attention as a sustainable novel protein source in fish feed due to its high nutritional value and low environmental impacts. In the past decade, effects of the use of black soldier fly in aquafeeds have widely been studied in salmonids. A meta-analysis was conducted to compile and systematically quantify the effect of black soldier fly in diets for salmonids on growth performance and nutrient utilization. The main meta-analysis showed that dietary inclusion of black soldier fly did not compromise the specific growth rate, feed conversion ratio, feed intake, protein digestibility and protein efficiency ratio in salmonids. A meta-regression was conducted to explore the possible causes of variation in growth rate, feed conversion ratio and feed intake between the studies. Fish species, protein source(s) replaced and black soldier fly inclusion level were partially responsible for the variation in growth rate between the studies. The protein source(s) replaced and black soldier fly inclusion level partially explained the variation in feed conversion ratio and feed intake respectively. The sub-data sets sorted according to the replaced protein source(s) showed that replacing fishmeal by black soldier fly decreased growth rate and feed intake in salmonids, but replacing non-fishmeal sources improved growth rate and feed conversion. This strengthened the importance of the type of replaced protein source(s) when evaluating nutritional values of black soldier fly for salmonids. In conclusion, the present meta-analysis showed that black soldier fly is a promising protein source for salmonid feeds.

**KEYWORDS**

fishmeal replacement, insect meal, meta-analysis, meta-regression, nutrient utilization, salmonids

## 1 | INTRODUCTION

Insect meals have received an increasing attention in recent years as a sustainable protein source for aquafeeds,<sup>1</sup> because insects are able to utilize organic side streams, and the production does not require

any agricultural land, has low water usage and contributes to lower greenhouse gas emissions.<sup>2</sup> The approval of use of processed insects in aquafeeds by the European Commission (Regulation 2017/893/EC, 2017) further promotes upscaling of insects as a novel protein source. One of the most favourable insect species to be used in feed

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is black soldier fly (*Hermetia illucens* Linnaeus, 1758) (BSF).<sup>3</sup> BSF is a good source of protein, lipid and minerals.<sup>4</sup> Furthermore, BSF is capable of converting low-quality organic material efficiently into high-quality nutrients,<sup>5</sup> although the possibility of using low-quality organic material as a substrate for growing insects is still limited by the regulatory framework in Europe. BSF is a good candidate for large-scale production due to its high growth rate and feed conversion efficiency, potential to be reared on organic side streams and suitability for automation.<sup>3</sup> In addition to nutritive value, BSF also contains bioactive compounds such as chitin, lauric acid and antimicrobial peptides, which can have health beneficial effects in animals.<sup>6</sup>

With the identification of great potential of BSF as a sustainable novel protein source in fish feed, the effects of the use of BSF in diets for salmonids such as Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) have widely been studied, focusing on growth performance, nutrient utilization, gut microbiota,<sup>7,8</sup> gut health and immune responses.<sup>9,10</sup> In literature, the studies on growth performance and nutrient utilization reported high variation in success of fish responses to BSF in diets. The use of BSF in diets was shown to have no effects,<sup>11–14</sup> negative effects<sup>11,12,14</sup> or even positive effects<sup>15</sup> on growth performance and nutrient utilization in salmonids. Furthermore, previous studies showed dose-dependent responses in fish to increasing dietary BSF levels or protein replacement levels.<sup>11,14</sup> In a recent review, English et al.<sup>16</sup> also discussed the inconsistency of results obtained in different studies investigating the effects of the use of BSF in diets on salmonid growth performance and nutrient utilization. The diverse nature of BSF rearing, downstream processing and study designs makes it difficult to directly compare the reported results to draw a general conclusion and to determine the dose-dependent responses across the studies.

Meta-analysis is a method to compile and statistically analyse results from a number of individual studies addressing similar research questions and produce integrated and broader interpretations. Recently, this approach was used to examine the effect of replacing fishmeal with insect meals on specific growth rate (SGR) of fish<sup>17</sup> and to determine the nutritional value of insects in aquafeeds.<sup>18</sup> These meta-analyses included data for various insect species as well as various aquatic species. The results of these two studies further emphasized that the analysis of individual insect and aquatic species can be more meaningful than the generalized results across different insect and aquatic species. The previous reviews on the topic of the effects of the use of BSF in salmonid diets concentrated on summarizing scientific literature in a narrative and qualitative approach.<sup>16,19</sup> The effect of BSF in salmonid diets has not yet been evaluated using a quantitative meta-analysis based approach according to our knowledge. In addition, the reasons of the inconsistency in success associated with the use of BSF in diets are important to identify in order to optimize the use of BSF in salmonid diets and design experiments. According to our knowledge, none of the previous meta-analyses took into consideration factors such as fish species, feed processing techniques, type of protein source(s) replaced by insects and developmental stage of insects that can influence the fish response to dietary insects. Therefore, in the present study, a meta-analysis was

conducted to 1) systematically review and summarize data from previous studies to determine the effect of dietary BSF on SGR, feed conversion ratio (FCR), feed intake, apparent digestibility coefficient (ADC) of protein and protein efficiency ratio (PER) in salmonids and 2) identify the factors causing the variation in response of salmonids to the use of BSF in diet.

## 2 | MATERIALS AND METHODS

### 2.1 | Literature search and data set

The present meta-analysis was conducted adhering to the principles in the Cochrane Handbook for Systematic Reviews of Interventions<sup>20</sup> and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.<sup>21</sup> A systematic literature search was conducted in ISI WEB OF KNOWLEDGE (1945–2021) and SCOPUS (1939–2021) on 11–15 March 2021, using the following search terms in combination with Boolean operators: insect; insects; black soldier fly; *H. illucens*; salmon; Atlantic salmon; *S. salar*; Coho salmon; *Oncorhynchus kisutch*; Chinook salmon; *Oncorhynchus tshawytscha*; trout; rainbow trout and *O. mykiss*. The terms were used to search Topic in ISI WEB OF KNOWLEDGE and Title-Abstract-Keywords in SCOPUS. The literature search strategy was supplemented with manual searches.

The selection process of studies to be included in the meta-analysis data set is shown in Figure 1. To prevent selection bias, following prespecified inclusion criteria were used: 1) The presence of a control group that did not include BSF; 2) protein source(s) in the control diet replaced by BSF; 3) studies investigated the effects of BSF on the growth performance (SGR, FCR and/or feed intake) or nutrient utilization (ADC of protein and PER) in salmonids. A study was considered as a growth study if the fish were fed for minimum 7 weeks or the fish at least doubled in weight during the feeding period; 4) reported standard deviation or standard error mean and 5) written in English. In addition, the data set included studies with nutrient balanced (major nutrients and/or amino acids) experimental diets to avoid the nutrient imbalances interference with the results. Duplicate reports, reviews and conference proceedings were not included. If a study contained more than one control diet, relevant BSF diets were compared separately with each individual control. When a study contained more than two treatments providing more than one comparison to the meta-analysis, the comparisons were individually coded.

Relevant data were extracted from each study using a standardized pro forma. Data extracted included: growth performance and/or nutrient utilization parameters including SGR, FCR, feed intake, ADC of protein and PER (calculated based on Equations (1), (2), (3) and (4)), number of experimental units per treatment, salmonid species, life stage of salmon, final body weight of fish, feed production method, type of protein source(s) replaced by BSF, developmental stage of BSF, processing method of BSF, dietary inclusion level of BSF, dietary chitin level and fishmeal replacement level. The fishmeal replacement level of BSF diets was calculated as [(Fish meal in

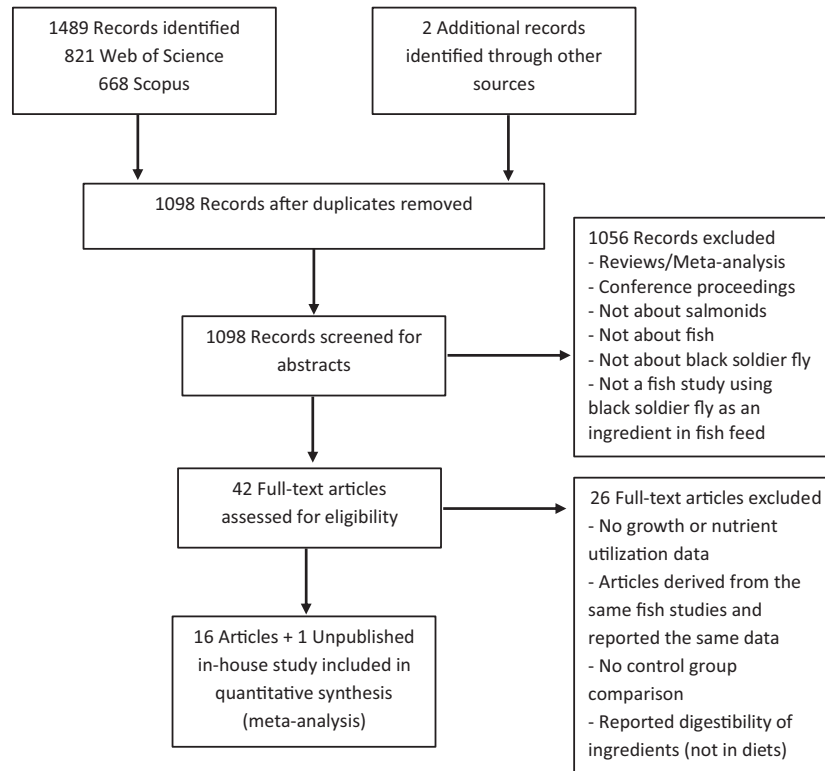


FIGURE 1 Selection of studies to be included in the meta-analysis data set. The selected 16 studies<sup>11-15,28,31,37,43,54,71,81,94-97</sup>

the control diet (%) – Fishmeal in the BSF diet (%)/Fishmeal in the control diet (%) × 100. In addition, the standard deviation or standardized error mean of SGR, FCR, feed intake, ADC of protein or PER from control and BSF diets-fed groups were extracted. Feed intake values were reported in numerous ways in the studies. Therefore, the feed intake values per fish per day were calculated using available information in reported studies, and the standard deviations of calculated feed intake values were determined using the prognostic method described by Ma et al.<sup>22</sup>

$$\text{SGR} = \frac{\ln \text{FBW} - \ln \text{IBW}}{t} \times 100, \quad (1)$$

where FBW = final body weight, IBW = initial body weight,  $t$  = number of days.

$$\text{FCR} = \frac{\text{FI}}{\text{BWG}} \quad (2)$$

where FI = feed intake, BWG = body weight gain.

$$\text{ADC of protein} = \left( 1 - \frac{M_D}{M_F} \times \frac{P_F}{P_D} \right) \times 100 \quad (3)$$

where  $M_D$  = marker concentration in diet,  $M_F$  = marker concentration in faeces,  $P_F$  = protein concentration in faeces,  $P_D$  = protein concentration in diet.

$$\text{PER} = \frac{\text{BWG}}{\text{PI}} \quad (4)$$

where BWG = body weight gain, PI = protein intake.

## 2.2 | Statistical analysis

The differences in growth performance and nutrient utilization parameters between control diets and BSF diets-fed fish within studies were calculated using a standardized effect size; Hedges'  $g$  (calculated based on Equations (5), (6) and (7)). The Hedges'  $g$  corrects for bias with small sample sizes and produces a statistical standardization of the findings for each study.<sup>23</sup>

The Hedges'  $g$  was calculated as:

$$\frac{\bar{X}_B - \bar{X}_C}{S_p} \quad (5)$$

where  $\bar{X}$  is the mean for the BSF (B) and control (C) groups,  $S_p$  is the pooled sample standard deviation and  $J$  is a correction factor for bias with small sample sizes.

The pooled standard deviation ( $S_p$ ) was calculated as:

$$S_p = \sqrt{\frac{(n_B - 1) SD_B^2 + (n_C - 1) SD_C^2}{n_B + n_C - 2}} \quad (6)$$

The correction factor ( $J$ ) was calculated as:

$$J = 1 - \frac{3}{4(n_B + n_C - 2) - 1} \quad (7)$$

where  $n$  is the sample size and SD is the standard deviation of the BSF (B) and control (C) groups.

The meta-analysis was performed in comprehensive meta-analysis version 3 software (Biostat Inc.) using random effects models to account for the variation among the populations of studies. The presence of true heterogeneity between the studies was identified with Cochran's Q-test<sup>24</sup> and the proportion of observed heterogeneity caused by true effects was quantified using  $I^2$  statistics.<sup>25</sup> When significant heterogeneity was detected, meta-regression analysis was conducted to explore the possible causes of heterogeneity. The categorical variables including fish species (Atlantic salmon vs. rainbow trout), feed production method (extrusion vs. pelleting), type of protein source(s) replaced (fishmeal vs. fishmeal + plant protein sources vs. non-fishmeal), BSF development stage (larvae vs. prepupae/pupae) and BSF processing method (full fat vs. defatted) and two continuous variables including dietary inclusion level of BSF and fish body size were included in the meta-regression analysis. Further differentiations of other variables were not possible due to the limited number or lack of data for carrying out a meaningful analysis. Meta-regression was not conducted with ADC of protein and PER data due to lack of data points in at least one group of each categorical variable. In addition, meta-analysis was conducted in sub-data sets sorted from the full data set according to the fish species, life stage of salmon, type of protein source(s) replaced, BSF development stage, BSF processing method and feed production method. Estimated effect sizes were visually displayed in forest plots created with comprehensive meta-analysis version 3 software (Biostat).

Linear and quadratic regression analyses between dietary inclusion level of BSF, dietary chitin level or fishmeal replacement level and effect sizes of SGR, FCR, feed intake, ADC of protein or PER were performed using IBM SPSS Statistics 27 software (IBM Corp.). The graphs were created using GraphPad Prism 9.0.0 software (GraphPad Software).

The chosen level of significance was  $p < 0.05$  and threshold level of tendency was  $p < 0.1$ . The possible publication bias was not conducted in the present study due to the occurrence of substantial heterogeneity with all outcomes, which may lead to false-positive claims for publication bias.<sup>26</sup>

### 3 | RESULTS

The nutritional compositions of BSF larvae, prepupae and pupae are shown in Table 1. The BSF on average contains 36–39% protein and 28–34% lipid. The amino acid profiles of BSF differ from mealworm (*Tenebrio molitor*) and house fly (*Musca domestica*) (Table S1). The BSF contains lower content of most of the essential amino acids than fishmeal, especially lysine (5.6–6.4% of protein) and methionine (1.7% of protein) (7.8 and 3% of protein, respectively, in fishmeal) (Table S2). The methionine content in BSF is superior to that in soy protein (1.4% of protein) (Table S2). The essential amino acid profile shows that BSF in general meets the amino acid requirements of Atlantic salmon and rainbow trout<sup>27</sup> (Table S2), except for lysine and methionine. However, the values in NRC<sup>27</sup> are more than a decade

old and advances in genetic and breeding programmes over the years have changed the nutritional requirements of salmonids.

The meta-analysis data set consisted of 16 publications in peer-reviewed journals and one unpublished in-house study (Table S3). Amongst these studies, 13 studies reported the nutritional composition of used BSF ingredients (Table S4). The studies were reported between 2007 and 2021. Either BSF larvae, prepupae or pupae were used in these studies in full-fat or defatted form. The BSF was included in the experimental diets by replacing traditional protein sources such as fishmeal, plant protein sources and/or animal protein sources, and the BSF inclusion levels in diets ranged from 5% to 60%. The sample sizes of studies ranged from two to four experimental units/treatments.

Amongst the selected studies, 13 studies were used to conduct the meta-analysis for SGR (36 comparisons) and all the 17 studies were used for FCR (49 comparisons) and feed intake (50 comparisons). The most studied salmonid species was rainbow trout (i.e. 11 studies) accounting for 50%, 53% and 54% of the SGR, FCR and feed intake data, respectively, whereas Atlantic salmon accounted for 50%, 47% and 46% of the SGR, FCR and feed intake data respectively. Amongst Atlantic salmon, four studies used pre-smolts and two studies used post-smolts. Seven studies (19 comparisons) were used to conduct the meta-analysis for ADC of protein. Atlantic salmon accounted for 68% of the ADC of protein data, whereas rainbow trout accounted for 32% of the data. Although two additional studies reported ADC of protein, one study was excluded from the analysis being an outlier as it gave extreme effect sizes,<sup>28</sup> and the other one was excluded because ADC of protein was reported as a graphical presentation.<sup>13</sup> Eight studies (21 comparisons) were used to conduct the meta-analysis for PER. Atlantic salmon accounted for 71% of the PER data, whereas rainbow trout accounted for 29% of the data.

#### 3.1 | Specific growth rate

The forest plot in Figure 2 shows the pooled effect of the use of BSF in diets on SGR in salmonids. In the full data set of SGR, the Hedges'  $g$  between BSF diets and control diets ranged from  $-5.71$  to  $8$ , with 78% of the comparisons showing an increase or no change in SGR in fish fed BSF diets compared to control diets. The meta-analysis of SGR showed a mean effect size of  $-0.014$  (Confidence interval:  $-0.615$  to  $0.586$ ). On average, SGR in salmonids fed BSF diets did not differ from those fed the control diets. The test of heterogeneity,  $Q$ -value was  $168.0$  with a corresponding  $p$  value of  $<0.001$ , showing a significant heterogeneity in true effect sizes of SGR between the studies. Furthermore, the  $I^2$  statistic showed that 79.2% of the observed heterogeneity was caused by the true effects rather than the sampling error. The variance of true effects ( $T^2$ ) was  $2.5$  and the standard deviation of true effects ( $T$ ) was  $1.6$ .

According to the meta-regression, fish species, protein source(s) replaced and dietary BSF level partially caused the heterogeneity

TABLE 1 Proximate (% dry matter), mineral (g kg<sup>-1</sup> dry matter), amino acid (% of crude protein) and fatty acid (% of total fatty acids) compositions of different developmental stages of black soldier fly (BSF)

Nutrient	Larvae		Prepupae		Pupae	
	Mean	SD	Mean	SD	Mean	SD
Dry matter (%)	32	5.3 (27)	35	5.9 (11)	40 (1)	
Crude protein	39	6.1 (58)	37	6.0 (16)	36	6.7 (2)
Crude lipid	28	8.7 (14)	34	9.2 (11)	28	17 (2)
Chitin	4.8	1.5 (9)	7.2	1.8 (9)	6.3 (1)	
Ash	10	4.5 (29)	10	4.6 (10)	14	7.4 (2)
<b>Minerals</b>						
Calcium	22	7.8 (12)	34	24 (5)	44 (1)	
Phosphorous	8.4	1.7 (17)	4.7	0.8 (5)	6.3 (1)	
Potassium	17	4.2 (12)	5.8	0.9 (5)	6.1 (1)	
Sodium	5	3.9 (12)	0.9	0.4 (5)	1.7 (1)	
Magnesium	3.8	1.3 (12)	2.8	0.6 (5)	3.7 (1)	
Manganese	0.2	0.05 (12)	0.2	0.1 (5)	0.4 (1)	
Iron	0.3	0.07 (12)	0.2	0.2 (5)	0.07 (1)	
Iodine	0.1	0.08 (10)				
Zinc	0.1	0.03 (11)	0.09	0.05 (4)	0.07 (1)	
<b>Essential amino acids</b>						
Arginine	4.8	0.6 (40)	4.8	1.1 (9)	5.5 (1)	
Histidine	2.7	0.5 (40)	3.0	0.7 (10)	3.4 (1)	
Isoleucine	4.2	0.7 (40)	4.2	0.9 (10)	4.7 (1)	
Leucine	6.6	0.8 (40)	6.6	1.4 (10)	7.8 (1)	
Lysine	5.9	1.0 (40)	5.6	1.3 (10)	6.4 (1)	
Methionine	1.7	0.3 (40)	1.7	0.4 (10)	1.7 (1)	
Phenylalanine	3.9	0.8 (40)	3.8	0.9 (10)	4.1 (1)	
Threonine	3.8	0.4 (40)	3.8	0.9 (10)	4.3 (1)	
Tryptophan	1.8	0.9 (21)	1.4	0.3 (6)	1.6 (1)	
Valine	5.8	0.7 (40)	5.8	1.3 (10)	6.8 (1)	
<b>Non-essential amino acids</b>						
Alanine	6.4	0.9 (35)	6.0	1.5 (8)	6.8 (1)	
Aspartic acid	8.6	0.9 (35)	8.3	2.2 (8)	11 (1)	
Glycine	5.1	0.6 (35)	5.1	1.3 (8)	6.5 (1)	
Glutamic acid	11	1.4 (34)	9.9	2.5 (8)	11 (1)	
Cysteine	0.8	0.7 (23)	1.1	1.0 (8)	0.8 (1)	
Tyrosine	5.7	1.4 (36)	5.9	2.0 (5)	6.8 (1)	
Proline	5.6	1.0 (34)	5.2	1.3 (8)	6.2 (1)	
Serine	4.2	0.4 (34)	4.1	1.5 (8)	4.7 (1)	
<b>Fatty acids</b>						
C12:0	37	9.9 (58)	43	11.9 (27)	65 (1)	
C14:0	7.5	1.4 (58)	6.9	2.0 (27)	9.7 (1)	
C16:0	16	3.1 (58)	13	3.6 (27)	8.6 (1)	
C16:1n7 (C16:1)	3.5	2.2 (55)	5.9	3.8 (27)	2.8 (1)	
C18:0	2.9	0.9 (58)	1.8	0.8 (27)	1.2 (1)	
18:1n9	13	4.3 (58)	12	5.0 (23)	6.8 (1)	

(Continues)

TABLE 1 (Continued)

Nutrient	Larvae		Prepupae		Pupae	
	Mean	SD	Mean	SD	Mean	SD
C18:2n6	14	6.7 (58)	6.7	3.4 (27)	5.2 (1)	
C18:3n3	1.5	0.8 (58)	3.9	6.9 (26)	0.7 (1)	
C20:4n6	0.4	0.5 (39)				
C20:5n3	1.1	1.5 (41)	0.1	0.1 (5)		
C22:6n3	0.7	0.9 (30)	0.1	0.2 (5)		

Note: Values in parentheses are the number of data points used for calculating the mean.

Sources for proximate and mineral compositions: BSF larvae,<sup>13,57-65</sup> BSF prepupae<sup>61,65-69</sup> and BSF pupae.<sup>60,65</sup> Sources for amino acid compositions: BSF larvae,<sup>11-13,28,49,54,57,64,65,70-80</sup> BSF pre-pupae<sup>37,65,66,69,80-82</sup> and BSF pupae.<sup>65</sup> Sources for fatty acid compositions: BSF larvae,<sup>11,13,54,57,58,62,65,83-89</sup> BSF pre-pupae<sup>65,67-69,90-93</sup> and BSF pupae.<sup>65</sup>

Abbreviation: SD, Standard deviation.

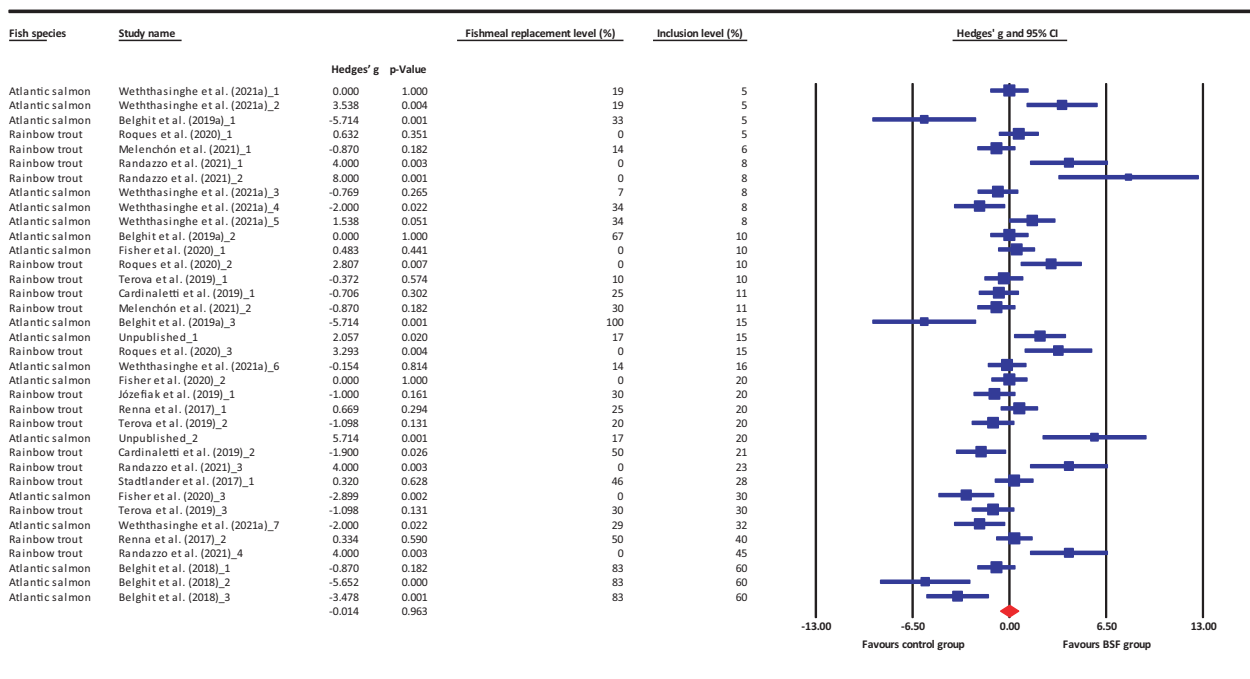


FIGURE 2 Forest plot of effect sizes (Hedges' g) of specific growth rate in salmonids between experimental diets containing black soldier fly (BSF) and control diets (full data set). The mean effect size, calculated according to a random effects model, is indicated by the red diamond at the bottom. The size of the blue squares illustrates the weight of each study relatively to the mean effect size. Smaller squares represent less weight. CI, Confidence interval

in SGR between the studies in the full data set. However, the other variables in the model including feed production method, BSF development stage, BSF processing method and fish body size did not explain heterogeneity at any significant level. The variables included in the meta-regression model could explain only 19% of the heterogeneity in SGR between studies (Table 2).

The meta-analyses of sub-data sets including salmon, salmon pre-smolts, rainbow trout, full-fat BSF and defatted BSF showed that, on average, the SGR of fish fed BSF diets did not differ from that of fish fed control diets. Three groups were identified according to the source(s) of protein replaced: 1) fishmeal, 2) fishmeal and plant protein sources and 3) non-fishmeal protein sources. The meta-analyses within these three groups showed that replacing fishmeal with BSF

decreased SGR of salmonids, whereas replacement of both fishmeal and plant protein sources did not change SGR. The replacement of non-fishmeal protein sources with BSF even increased the SGR. The analyses further showed the presence of unexplained heterogeneity between the studies in all the sub-data sets. Nevertheless, there were no sufficient data to conduct further subgroup analyses or meta-regression in these data sets (Table 3).

Linear and quadratic regressions in the full data set and sub-data sets revealed no linear or quadratic relationships between the dietary inclusion level of BSF (Table S5) or dietary chitin level (Tables S6) and effect sizes of SGR. In the BSF larvae data set, however, SGR in salmonids tended ( $p = 0.088$ ) to decrease linearly with increasing level of BSF in the diet. The fishmeal replacement level

TABLE 2 Heterogeneity in effect sizes (Hedges' g) and significance level (p value) of different categorical and continuous variables determined by meta-regression analysis

Parameter	p Value									
	Heterogeneity explained by the model (%)	Test of the model	Heterogeneity unexplained by the model	Fish species	Feed production	Type of protein source replaced	BSF development stage	BSF processing method	BSF inclusion level	Fish body size
SGR	0.19	***	***	**	NS	***	NS	NS	*	NS
FCR	0.02	**	***	NS	NS	**	NS	NS	NS	NS
Feed intake	-0.09	*	***	NS	NS	NS	NS	NS	*	NS

Abbreviations: BSF, Black soldier fly (*Hermetia illucens*); FCR, feed conversion ratio; SGR, specific growth rate. Asterisks denote level of significance (NS: not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

had negative linear relationships and/or quadratic relationships with effect sizes of SGR in the full data set, as well as in sub-data sets including salmon, rainbow trout, BSF larvae, BSF prepupae/pupae and defatted BSF (Figure 3 and Table S7).

### 3.2 | Feed conversion ratio

The forest plot in Figure 4 shows the pooled effect of BSF inclusion in diets on FCR in salmonids. In the full data set of FCR, the Hedges' g, between BSF diets and control diets ranged from -8 to 4.8, with 80% of the comparisons showing a decrease or no change in FCR in fish fed BSF diets compared to control diets. The meta-analysis of FCR showed a mean effect size of 0.094 (Confidence interval: -0.341 to 0.529). On average, FCR in salmonids fed BSF diets did not differ from those fed the control diets. The test of heterogeneity, Q-value was 185.2 with a corresponding p value of <0.001, showing a significant heterogeneity in true effect sizes of FCR between the studies. Furthermore, the  $I^2$  statistic showed that 74.1% of the observed heterogeneity was caused by the true effects rather than the sampling error. The variance of true effects ( $T^2$ ) was 1.7 and the standard deviation of true effects (T) was 1.3.

According to the meta-regression, the protein source(s) replaced partially caused the heterogeneity in FCR between the studies in the full data set. However, the other variables in the model including fish species, feed production method, BSF development stage, BSF processing method, BSF inclusion level and fish body size did not explain heterogeneity at any significant level. The variables included in the meta-regression model could explain only 2% of the heterogeneity in FCR between studies (Table 2).

The salmon data set showed that, on average, dietary inclusion of BSF tended ( $p = 0.095$ ) to increase FCR in salmon compared to control diets. The mean effect size for salmon pre-smolts showed an increased FCR in fish fed BSF diets compared to fish fed control diets. The meta-analysis of the FCR in rainbow trout group showed no statistically significant effect of BSF in the diet. The meta-analysis of the three groups categorized according to the type of protein source(s) replaced showed that replacing fishmeal with BSF did not change FCR of salmonids, whereas the replacement of both fishmeal and plant protein sources increased FCR and the replacement of non-fishmeal protein sources decreased FCR. The two sub-data sets sorted according to the processing method of BSF showed that feeding either full-fat or defatted BSF had no impact on FCR in salmonids. Although all the sub-data sets revealed the presence of unexplained heterogeneity between the studies, potential factors responsible for this could not be identified due to insufficient availability of data (Table 3).

Linear and quadratic regressions in the full data set and sub-data sets revealed that there were no linear or quadratic relationships between the inclusion level of BSF (Table S5) or chitin level in the diet (Table S6) and effect sizes of FCR. In the salmon data set, however, FCR tended ( $p = 0.05$ ) to increase linearly with increasing level of BSF in the diet. Fishmeal replacement level on the other hand

**TABLE 3** Effect sizes (Hedges'  $g$ ) of growth performance and nutrient utilization data in salmonids between experimental diets containing black soldier fly (BSF) and control diets (sub-data sets)

Parameter	Data subset	Number of studies	Number of comparisons	Random effect model			Heterogeneity		
				Hedges' $g$	95% CI	$p$	$Q$	$p$	$I^2$
SGR	Species								
	Atlantic salmon	5	18	-0.72	-1.7 to 0.2	NS	91.9	***	81.5
	Rainbow trout	8	18	0.60	-0.2 to 1.4	NS	72.5	***	76.5
	Salmon life stage								
	Pre-smolt	4	15	-0.36	-1.3 to 0.6	NS	73.4	***	80.9
	Post-smolt	No sufficient data							
	Protein source(s) replaced								
	Fishmeal	7	14	-0.76	-1.4 to -0.2	*	29.7	**	56.2
	Fishmeal + Plant protein	3	12	-0.25	-1.4 to 0.9	NS	64.1	***	82.8
	Non-fishmeal	3	10	1.98	0.5 to 3.4	**	51.2	***	82.4
	BSF processing method								
	Full-fat	6	16	-0.37	-1.1 to 0.3	NS	50.3	***	70.2
	Defatted	8	20	0.26	-0.7 to 1.2	NS	113	***	83.1
FCR	Species								
	Atlantic salmon	6	23	0.51	-0.1 to 1.1	NS	77.4	***	71.6
	Rainbow trout	11	26	-0.31	-0.9 to 0.3	NS	104	***	76.1
	Salmon life stage								
	Pre-smolt	4	15	1.01	0.3 to 1.7	**	47.6	***	70.6
	Post-smolt	No sufficient data							
	Protein source(s) replaced								
	Fishmeal	11	27	0.18	-0.3 to 0.6	NS	71.4	***	63.6
	Fishmeal + Plant protein	3	12	1.11	0.3 to 1.9	**	36.8	***	70.1
	Non-fishmeal	3	10	-1.89	-3.3 to -0.5	**	49.2	***	81.7
	BSF processing method								
	Full-fat	9	25	0.37	-0.1 to 0.9	NS	74.2	***	67.7
	Defatted	10	24	-0.30	-1.0 to 0.4	NS	109	***	78.9
Feed intake	Species								
	Atlantic salmon	6	23	-0.01	-0.5 to 0.5	NS	55.7	***	60.5
	Rainbow trout	11	27	-0.18	-0.7 to 0.4	NS	90.6	***	71.3
	Salmon life stage								
	Pre-smolt	4	15	0.08	-0.3 to 0.5	NS	21.2	NS	33.9
	Post-smolt	No sufficient data							
	Protein source(s) replaced								
	Fishmeal	11	28	-0.70	-1.3 to -0.1	*	103	***	73.9
	Fishmeal + Plant protein	3	12	0.12	-0.4 to 0.7	NS	21.1	*	48.0
	Non-fishmeal	3	10	0.51	-0.1 to 1.1	NS	16.4	NS	45.0
	BSF processing method								
	Full-fat	9	25	-0.49	-1.1 to 0.1	NS	82.2	***	70.8

(Continues)



TABLE 3 (Continued)

Parameter	Data subset	Number of studies	Number of comparisons	Random effect model			Heterogeneity		
				Hedges' g	95% CI	p	Q	p	I <sup>2</sup>
ADC of protein	Defatted	10	25	0.21	-0.3 to 0.7	NS	62.1	***	61.4
	Species								
	Atlantic salmon	4	13	-1.03	-1.7 to -0.4	**	28.7	**	58.2
	Rainbow trout	No sufficient data							
	BSF development stage								
	Larvae	6	16	-0.73	-1.4 to -0.1	*	45.2	***	66.8
	Prepupae/pupae	No sufficient data							
	BSF processing method								
	Full-fat	3	11	-0.52	-1.4 to 0.3	NS	36.7	***	72.2
	Defatted	No sufficient data							
Feed production method									
Extrusion	5	15	-0.79	-1.5 to -0.1	*	44.4	***	68.5	
Pelleting	No sufficient data								
PER	Species								
Atlantic salmon	4	15	-0.19	-1.0 to 0.7	NS	63.1	***	77.8	
Rainbow trout	No sufficient data								
BSF processing method									
Full-fat	5	14	-0.33	-1.0 to 0.4	NS	41.2	***	68.5	
Defatted	No sufficient data								

Abbreviations: ADC of protein, apparent digestibility of protein; CI, Confidence interval; FCR, feed conversion ratio; PER, protein efficiency ratio; SGR, specific growth rate.

Asterisks denote level of significance (NS, not significant, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

had both positive linear relationships and quadratic relationships with the effect size of FCR in the full data set, and the sub-data sets including rainbow trout, BSF prepupae/pupae and defatted BSF (Figure 5 and Table S7).

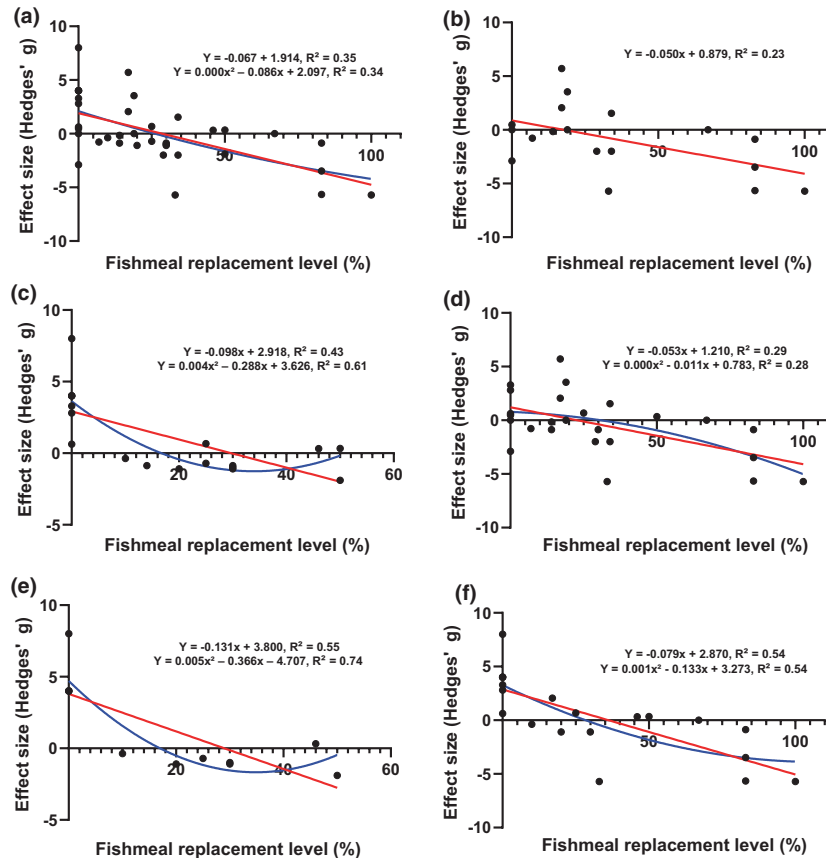
### 3.3 | Feed intake

The forest plot in Figure 6 shows the pooled effect of BSF inclusion in diets on feed intake in salmonids. In the full data set of feed intake, the Hedges' g, between BSF diets and control diets ranged from -27 to 4.5, with 78% of the comparisons showing an increase or no change in feed intake in fish fed BSF diets compared to control diets. The meta-analysis of feed intake showed a mean effect size of -0.099 (Confidence interval: -0.476 to 0.277). On average, the feed intake in salmonids fed BSF diets did not differ from those fed the control diets. The test of heterogeneity, Q-value was 146.4 with a corresponding p value of <0.001, showing a significant heterogeneity in true effect sizes of feed intake between the studies. Furthermore, the I<sup>2</sup> statistic showed that 66.5% of the observed

heterogeneity was caused by the true effects rather than the sampling error. The variance of true effects (T<sup>2</sup>) was 1.1 and the standard deviation of true effects (T) was 1.1.

According to the meta-regression, BSF inclusion level partially caused the heterogeneity in feed intake between the studies in the full data set. However, the other variables in the model did not explain heterogeneity at any significant level (Table 2). The sub-data sets showed that, on average, feed intake did not differ between fish fed BSF diets and control diets, except replacing fishmeal with BSF decreased feed intake and replacing non-fishmeal protein sources tended ( $p = 0.095$ ) to increase feed intake of salmonids (Table 3).

Linear and quadratic regressions in the full data set and sub-data sets revealed that there were no linear or quadratic relationships between the inclusion level of BSF (Table S5) or chitin level in the diet (Table S6) and effect sizes of feed intake. Fishmeal replacement level on the other hand had negative linear relationships and/or quadratic relationships with the effect size of feed intake in the full data set, and the sub-data sets including salmon, BSF larvae, full-fat BSF and defatted BSF (Figure 7 and Table S7).



**FIGURE 3** The relationship between the fishmeal replacement level by black soldier fly and the effect sizes (Hedges' g) of specific growth rate for the full data set (a), salmon data set (b), rainbow trout data set (c), black soldier fly larvae data set (d), black soldier fly prepupae/pupae data set (e) and defatted black soldier fly data set (f). Red lines represent linear relationships and blue lines represent quadratic relationships

### 3.4 | Protein digestibility

The forest plot in Figure 8 shows the pooled effect of BSF inclusion in diets on ADC of protein in salmonids. In the full data set, the Hedges' g, between BSF diets and control diets ranged from  $-3.30$  to  $1.43$ , with 74% of the comparisons showing no change in ADC of protein in fish fed BSF diets compared to control diet. The meta-analysis of ADC of protein in the full data set showed a mean effect size of  $-0.540$  (Confidence interval:  $-1.096$  to  $0.017$ ). On average, the dietary inclusion of BSF tended ( $p = 0.057$ ) to decrease ADC of protein in salmonids compared to control diets. The test of heterogeneity, Q-value was 49.3 with a corresponding  $p$  value of  $<0.001$ , showing a significant heterogeneity in true effect sizes of ADC of protein between the studies. Furthermore, the  $I^2$  statistic indicates that 63.5% of the observed heterogeneity was caused by the true effects rather than the sampling error. The variance of true effects ( $T^2$ ) was 0.9 and the standard deviation of true effects ( $T$ ) was 1.0.

For salmon, BSF larvae and extruded feed data sets, the mean effect sizes of ADC of protein showed that dietary inclusion of BSF decreased ADC of protein compared to control diets. The full-fat BSF data set showed no difference in ADC of protein between BSF and control groups (Table 3). There were no sufficient data available for other subgroups to conduct meta-analysis.

Linear and quadratic regressions in the full data set revealed that there were no significant linear or quadratic relationships between the dietary inclusion level of BSF (Tables S5), dietary chitin level (Tables S6) or fishmeal replacement level (Table S7) and effect sizes of ADC of protein.

### 3.5 | Protein efficiency ratio

The forest plot in Figure 9 shows the pooled effect of BSF inclusion in diets on PER in salmonids. In the full data set of PER, the Hedges' g, between BSF diets and control diets ranged from  $-4.8$  to  $3.4$ , with 76% of the comparisons showing an increase or no change in PER in fish fed BSF diets compared to control diet. The meta-analysis of PER in the full data set showed a mean effect size of  $-0.064$  (Confidence interval:  $-0.655$  to  $0.526$ ). On average, the PER in salmonids fed BSF diets did not differ from those fed the control diets. The test of heterogeneity, Q-value was 68.9 with a corresponding  $p$  value of  $<0.001$ , showing a significant heterogeneity in true effect sizes of PER between the studies. Furthermore, the  $I^2$  statistic showed that 71% of the observed heterogeneity was caused by the true effects rather than the sampling error. The variance of true effects ( $T^2$ ) was 1.3 and the standard deviation of true effects ( $T$ ) was 1.1.

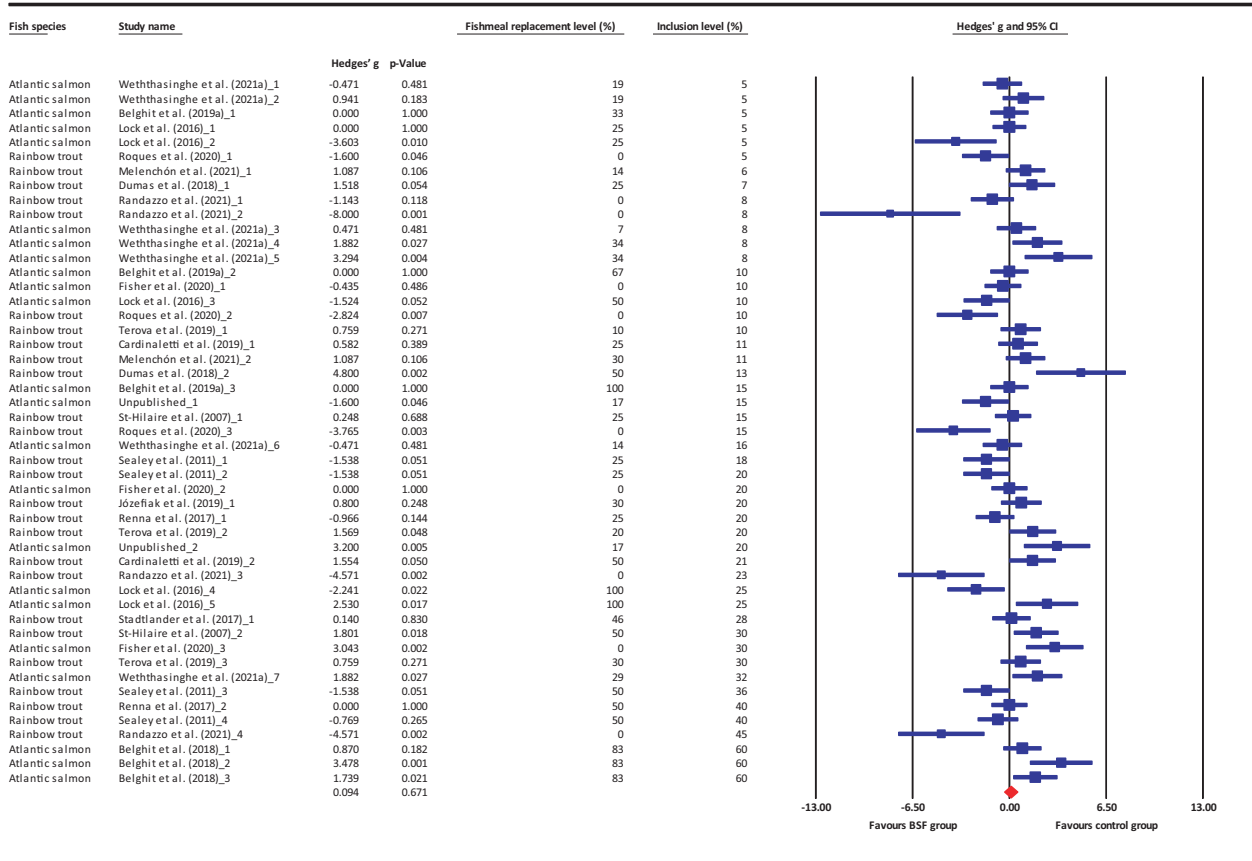


FIGURE 4 Forest plot of effect sizes (Hedges' g) of feed conversion ratio in salmonids between experimental diets containing black soldier fly (BSF) and control diets (full data set). The mean effect size, calculated according to a random effects model, is indicated by the red diamond at the bottom. The size of the blue squares illustrates the weight of each study relatively to the mean effect size. Smaller squares represent less weight. CI, Confidence interval

For salmon and full-fat BSF data sets, the mean effect sizes of PER showed that dietary inclusion of BSF had no impact on PER compared to control diets (Table 3). There were no sufficient data available for other groups to conduct meta-analysis.

Linear and quadratic regressions in the full data set revealed that there were no significant linear or quadratic relationships between the dietary inclusion level of BSF (Tables S5), dietary chitin level (Tables S6) or fishmeal replacement level (Table S7) and effect sizes of PER.

#### 4 | DISCUSSION

The present meta-analysis provided an overall insight into the direction of effects obtained across studies that used BSF in diets for Atlantic salmon and rainbow trout. The control diets in the present data set contained fishmeal, plant and land animal protein sources. Majority of the individual BSF containing experimental diets in the full data set (>75%) of the present meta-analysis supported similar or superior growth rate, feed utilization and feed intake in salmonids compared to the respective control diets. However, the mean effect sizes for the full data set revealed no differences in growth rate and

feed conversion between the fish fed BSF diets and control diets. This was accompanied by the mean effect size for feed intake, showing no difference between BSF and control diets-fed fish. Hence, the use of 5–60% BSF in salmonid diets replacing fishmeal, plant and animal protein is possible without compromising growth performance. It is possible that the mean effect sizes in the meta-analysis averaged out the possible factors influencing the effectiveness of dietary BSF in salmonids.<sup>17</sup> The wide range of effect sizes of SGR, FCR and feed intake in the present analysis indicated the variation in the effectiveness of the use of BSF meal in diets for salmonids. The heterogeneity test also confirmed the possible effect of influencing factors and meta-regression was used to identify these factors in the present study.

Previous reviews showed that different fish species responded differently to dietary BSF.<sup>19,29</sup> In accordance, the present study also showed that the type of salmonid species was partially responsible for the heterogeneity of growth rate in salmonids between the studies. This confirmed the importance of conducting meta-analysis for salmon and rainbow trout separately. Separate meta-analyses for each fish species also showed that the use of BSF in diets in the form of either full-fat or defatted had no impact on growth rate, feed utilization and feed intake. However, further analysis revealed that the dietary

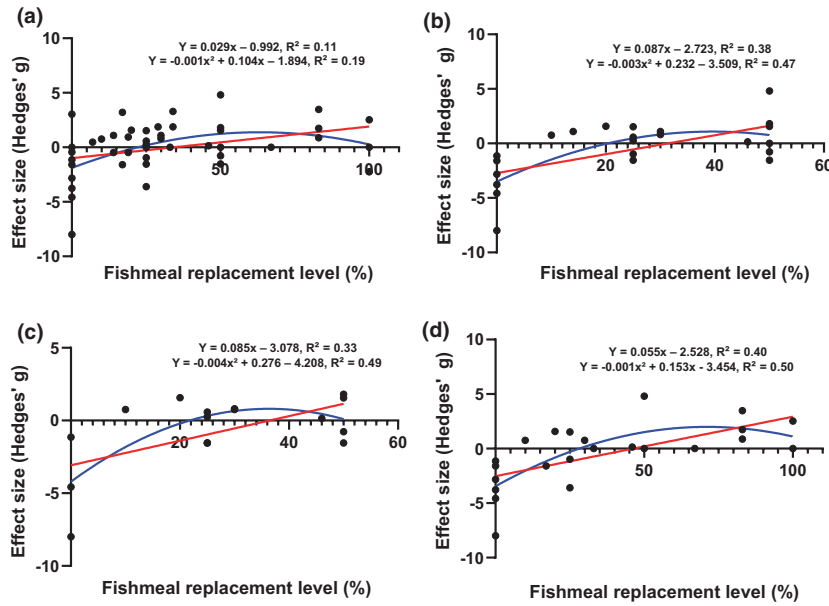


FIGURE 5 The relationship between the fishmeal replacement level by black soldier fly and the effect sizes (Hedges' g) of feed conversion ratio for the full data set (a), rainbow trout data set (b), black soldier fly prepupae/pupae data set (c) and defatted black soldier fly data set (d). Red lines represent linear relationships and blue lines represent quadratic relationships

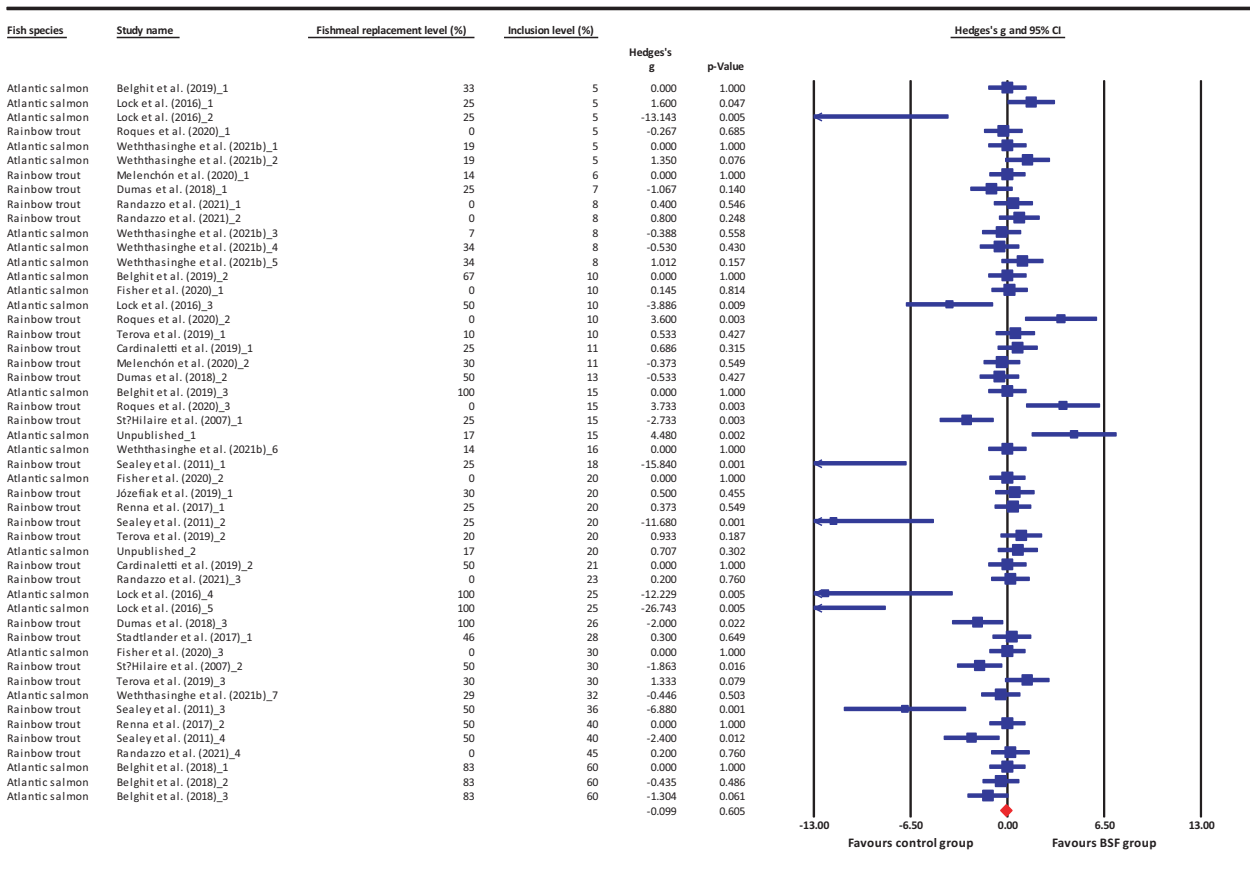


FIGURE 6 Forest plot of effect sizes (Hedges' g) of feed intake in salmonids between experimental diets containing black soldier fly (BSF) and control diets (full data set). The mean effect size, calculated according to a random effects model, is indicated by the red diamond at the bottom. The size of the blue squares illustrates the weight of each study relatively to the mean effect size. Smaller squares represent less weight. CI, Confidence interval

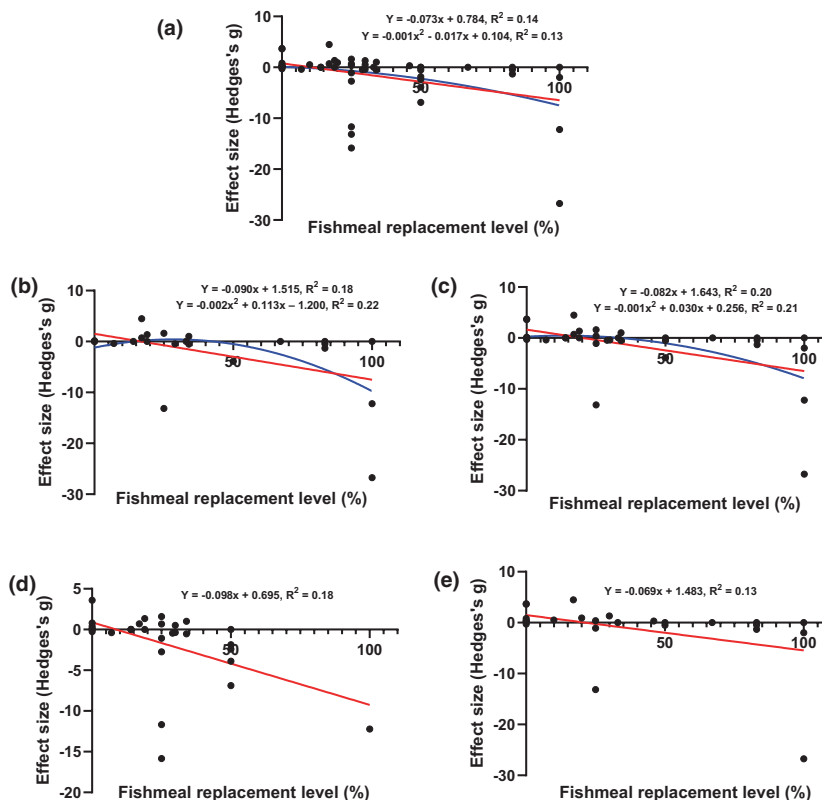


FIGURE 7 The relationship between the fishmeal replacement level by black soldier fly and the effect sizes (Hedges' g) of feed intake for the full data set (a), salmon data set (b), black soldier fly larvae data set (c), full-fat black soldier fly data set (d) and defatted black soldier fly data set (e). Red lines represent linear relationships and blue lines represent quadratic relationships

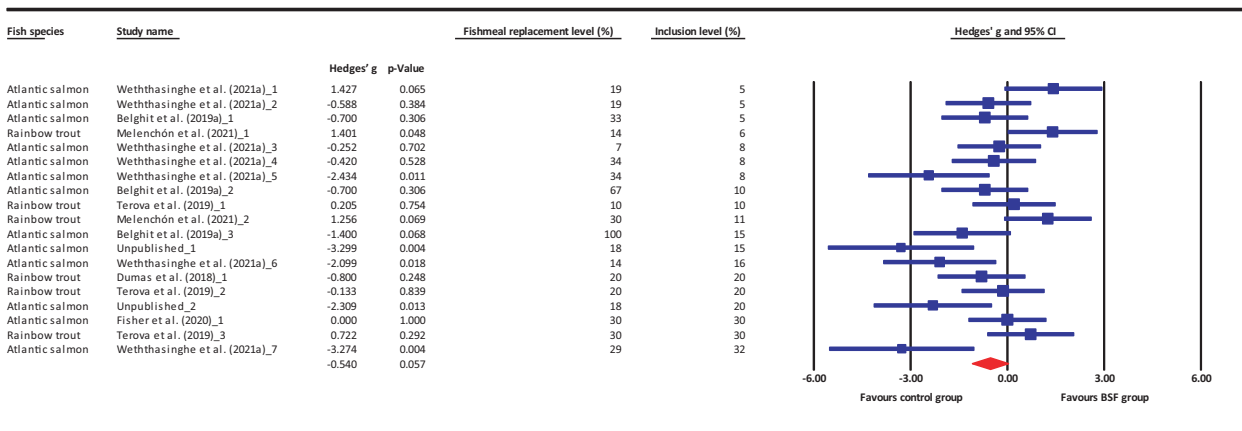
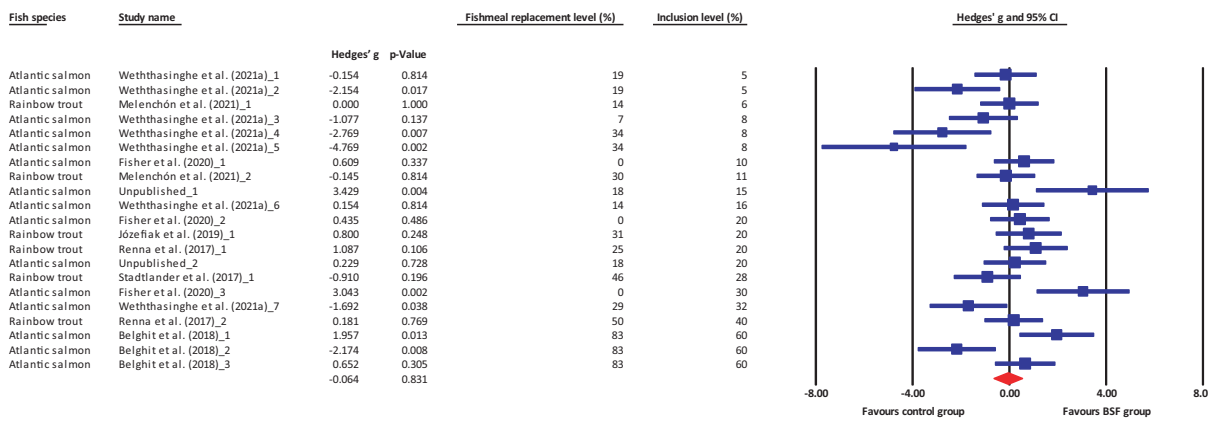


FIGURE 8 Forest plot of effect sizes (Hedges' g) of apparent digestibility coefficient of protein in salmonids between experimental diets containing black soldier fly (BSF) and control diets (full dataset). The mean effect size, calculated according to a random effects model, is indicated by the red diamond at the bottom. The size of the blue squares illustrates the weight of each study relatively to the mean effect size. Smaller squares represent less weight. CI, Confidence interval

inclusion of BSF depressed the feed utilization in salmon pre-smolts, indicating low utilization of BSF for salmon reared in freshwater.

As shown in Table S8, the nutrient composition of BSF varies with the processing methods. In a recent review, English et al.<sup>16</sup> also showed that the quality and nutritional composition of BSF can change dramatically based on processing. The processing method

of insect meals is a crucial point that can have a direct effect on the growth performance and feed efficiency in fish.<sup>30</sup> In a previous study, growth performance of salmon differed by how the BSF was processed.<sup>31</sup> In addition, Basto et al.<sup>32</sup> reported that defatted BSF meal improved the digestibility of protein and amino acids compared to full-fat meal in European sea bass (*Dicentrarchus labrax*)



**FIGURE 9** Forest plot of effect sizes (Hedges' g) of protein efficiency ratio in salmonids between experimental diets containing black soldier fly (BSF) and control diets (full data set). The mean effect size, calculated according to a random effects model, is indicated by the red diamond at the bottom. The size of the blue squares illustrates the weight of each study relatively to the mean effect size. Smaller squares represent less weight. CI, Confidence interval

juveniles. On the contrary, present results showed that processing method of BSF (full-fat vs. defatted) did not cause heterogeneity in the growth performance of salmonids between the studies. The growth performances of salmonids fed BSF, on average, were similar to those fed control diets despite the BSF was in full-fat or defatted form. The meta-analysis conducted by Hua<sup>17</sup> showed that full-fat and defatted BSF meals affect SGR of fish similarly when only the nutrient-balanced diets were included in the data set. Although present and previous meta-analyses showed no differences in fish responses to the use of defatted and full-fat BSF in the diet, these results should be interpreted with caution because the diets in the data sets contained BSF with varying degrees of defatting (partially or fully defatted).

Feed production technologies such as extrusion might affect the nutritional values of feeds containing insect meals.<sup>17</sup> The processing method of feed (pelleting vs. extrusion) was reported to affect the responses of fish to dietary changes.<sup>33-35</sup> For instance, fish fed extruded diets had higher weight gain at low dietary protein levels<sup>34</sup> and higher nutrient utilization<sup>35</sup> than pelleted feeds. When the low-fishmeal diets were supplemented with enzymes, pelleted feed, but not extruded feed, improved fish growth and nutrient utilization.<sup>33</sup> On the contrary, present results showed that the feed production method did not explain the heterogeneity of SGR, FCR and feed intake in fish fed BSF across the studies. Additionally, the developmental stage of BSF did not contribute to the heterogeneity between the studies, even though the nutrient composition varied with the developmental stage of BSF. This indicates that differences in nutrient composition during BSF stages may not be sufficiently large enough to have an impact on fish growth performance.

The meta-analysis conducted by Hua<sup>17</sup> demonstrated that the use of up to 29% BSF meals in diets had no adverse effect on fish growth rate in comparison with control diets with similar nutrient content, but decreased at higher levels. Liland et al.<sup>18</sup> observed a

linear reduction in SGR of fish and shellfish species used in aquaculture with increasing BSF level in the diet. In the present study, the growth performance parameters in salmonids did not show any linear or quadratic relationships with BSF inclusion level. Furthermore, there was no clear breaking point detected for both SGR and FCR with the increasing level of BSF in the diet of salmonids (data not shown). Nevertheless, dietary level of BSF could partially explain the heterogeneity of SGR and feed intake in salmonids existing across the studies used. In addition, BSF larvae data set showed a tendency to reduce SGR linearly with increasing dietary BSF level. When only salmon was considered in the analysis, increasing dietary level of BSF also tended to increase FCR linearly ( $R^2 = 0.14$  and  $p = 0.05$ ).

As indicated by the meta-regression for growth performance parameters, the effects of the types of protein source(s) replaced by BSF is worth further investigation. Thus, meta-analysis was conducted in the sub-data sets sorted according to the protein source(s) replaced by BSF. The replacement of fishmeal by BSF negatively affected the growth rate and feed intake in salmonids but did not affect the FCR. Although the replacement of fishmeal and plant protein sources with BSF did not affect SGR and feed intake of salmonids, it increased FCR. The replacement of non-fishmeal protein sources with BSF even increased the growth rate, as well as reduced FCR in salmonids. Hence, the present results strengthened the importance of the type of protein source(s) replaced by BSF when evaluating the nutritional values of BSF in salmonids. The fishmeal replacement levels in the studies included in the present meta-analysis ranged from 0% to 100%. The linear regression analysis showed that the increasing fishmeal replacement by BSF negatively affected the SGR, FCR and feed intake in salmonids. All the sub-data sets, except the full-fat BSF data set, also showed linear decrease in SGR with increasing level of fishmeal replacement. Similarly, FCR in fish increased linearly with increasing level of fishmeal replacement in rainbow trout, defatted BSF and BSF prepupae/pupae data sets. Such linear reductions were also observed for feed intake in salmon,

BSF larvae, full-fat and defatted BSF data sets. As stressed by Hua and Bureau,<sup>36</sup> fishmeal replacement level might not be an objective parameter in evaluating nutritive values of alternative ingredients such as insect meals because the composition and nutritional value of fishmeal can vary widely. However, it can still provide a good indication on the dose response of fish for the replacement level of fishmeal in the diet.

All studies used in the present meta-analysis had balanced essential amino acid composition between the control and BSF diets, except one study that did not report any information regarding the amino acid profiles of diets or supplementation of amino acids.<sup>37</sup> Hence, the differences in fish responses according to the replaced protein source(s) were likely a reflection of the true differences between the nutritional values of the BSF and other protein sources rather than an artefact of discrepancies in the dietary amino acid profiles. It is possible that the depressed growth performance of salmonids fed diets replacing fishmeal might be due to limiting digestible amino acids in BSF compared to fishmeal. This illustrates the importance of determining the digestible protein, amino acids and energy levels in both the control diet and the test ingredient. The studies used in the present meta-analysis did not report any consideration of digestible amino acids in diet formulations. In literature, limited information is available on protein and amino acid digestibility of BSF in salmonids. The protein digestibility coefficient of BSF larvae meal was reported as 89% in Atlantic salmon<sup>12</sup> and 85% in rainbow trout.<sup>14</sup> Fisher et al.<sup>12</sup> further showed that the protein digestibility of BSF was lower than soybean meal (96%) and higher than corn protein concentrate (85%). In addition, Dumas et al.<sup>14</sup> reported that the digestibility of essential amino acids in BSF larvae meal varied from 84% to 96% in rainbow trout, while the digestibility of conditionally essential amino acid-like taurine was 57%. In these two studies, faeces were collected for digestibility estimation using faecal collection columns attached to the tanks. This might overestimate the protein digestibility compared to other faecal collection methods such as stripping, due to leaching of nitrogen (N) depending on the type of feed as explained by Shomorin et al.<sup>38</sup> Fishmeal may contain nutritional components that promote fish growth beyond the digestible nutrient content alone, such as taurine and low molecular weight compounds.<sup>39-41</sup> These components may be lacking in diets containing other protein sources and lead to better growth performance when such protein sources were replaced by BSF as explained in the review by Collins et al.<sup>42</sup> The growth reduction when fishmeal was replaced by BSF can also be due to decreased feed intake. However, the fishmeal replaced data set contained two studies which gave comparatively lower effect sizes for feed intake than the other studies.<sup>31,43</sup> It is, thus, possible that these two studies might influence the overall results for feed intake in this group. The improved growth in non-fishmeal replaced group can also be related to feed intake, as there was a tendency to increase in feed intake in this group.

As observed for growth performance data, the present study also showed that majority of the experimental diets containing

BSF in literature gave similar protein digestibility and PER in salmonids as the control diets. Furthermore, the meta-analysis showed that, on average, the dietary inclusion of BSF did not affect PER, but tended to decrease protein digestibility. Several sub-data sets also showed that the use of BSF decreased protein digestibility in salmonids compared to control diets, indicating negative effects of BSF on protein digestibility. The data sets consisted of different variables that can influence these results. In a review, English et al.<sup>16</sup> showed that the rearing substrate of BSF can be responsible for the inconsistency of nutrient digestibility of salmonids fed BSF between the studies. Moreover, similar to other animal protein sources such as fishmeal,<sup>44</sup> the drying method and temperature may have a large impact on the nutritional quality and protein digestibility of insect meals.<sup>45</sup> These influencing factors were not considered in the present study, and the comparison across the studies is thus complicated.

The exoskeleton of insects characteristically contains chitin.<sup>46</sup> The chitin content of the BSF ingredients in the present data set ranged from 3% to 17% (Table S4), and dietary chitin levels varied from 0.2% to 3% (dry matter basis) (Table S3). As previous studies suggested,<sup>11,47,48</sup> chitin can be the reason for observed negative effects (at least tend to) of BSF on growth performance and protein digestibility in several data sets in the present study. The dietary protein contents in the data set mostly covered the requirement of salmon and rainbow trout,<sup>27</sup> but the protein contents were calculated using the nitrogen-to-protein conversion factor of 6.25. Since BSF contains non-protein N from chitin, Janssen et al.<sup>49</sup> and Belghit et al.<sup>50</sup> have recently suggested that a factor between 4.2 and 5 might be more appropriate for BSF to avoid the overestimation of the protein content. Furthermore, chitin is poorly digestible in salmon and rainbow trout (13-40% and 2-5% respectively)<sup>51,52</sup> and can thus act as a filler in the diet.<sup>48</sup> Poorly digestible chitin can also increase faecal N excretion, and leads to overestimate the protein content in the faeces and underestimate the protein digestibility. This shows the importance of correcting the protein digestibility for chitin excreted as non-protein N with faeces, but none of the studies in the present meta-analysis reported a such correction. In addition, the chitin matrix in the exoskeleton of insects contains bound amino acids.<sup>53</sup> This might reduce the availability of protein in BSF for protease enzymes<sup>29</sup> or the activity of protease enzymes.<sup>13</sup> However, Basto et al.<sup>32</sup> reported that chitin alone cannot explain the lower nutrient digestibility in insect meals. Even though chitin can compromise protein digestibility, several studies reported no effect of the use of BSF meal on protein digestibility in both salmon<sup>12</sup> and rainbow trout.<sup>54</sup> The present regression analysis also showed no relationships between dietary chitin level and growth or nutrient utilization parameters. Nevertheless, this should be viewed with caution because only the data from six studies that reported chitin contents were used for these regression analyses.

In addition to chitin, other factors in the BSF, such as saturated fatty acids or other compounds, can also cause negative effects on fish growth. A previous meta-analysis showed that high saturated fatty acids (>39% of total fatty acids) and increasing level of

lauric acid in the diet decreased final body weight of fish fed BSF.<sup>18</sup> Nevertheless, two previous studies showed that dietary inclusion of BSF larvae oil (2.5–12%) did not affect the growth performance of salmon<sup>55</sup> and rainbow trout,<sup>14</sup> although the BSF oil diets in Belghit et al.<sup>55</sup> contained high levels of saturated fatty acids and lauric acid (48–51% and 22–29% of total fatty acids respectively). Lauric acid is also considered as a bioactive compound which can have health beneficial effects in animals,<sup>6</sup> but such effects of BSF lauric acid have not yet been verified in salmonids. Hence, more research is needed to confirm the impact of fatty acid profile of BSF on salmonid performance.

A meta-analysis implies limitations associated with diverse nature of studies, and interpretation of effect sizes obtained in a meta-analysis may be controversial, especially if number of relevant studies are limited.<sup>56</sup> The comparison across studies should ideally consider all biological and dietary factors.<sup>36</sup> Many of the variables that exist across studies were considered in the present study, but unexplained heterogeneity still existed among the studies even after considering these influencing factors. Other factors causing discrepancy among studies might be related to the quality of BSF, rearing substrates of BSF, nutrient composition of BSF, level of anti-nutritive as well as bioactive compounds in BSF, degree of defatting of BSF, drying method of BSF and temperatures, level of protein replaced by BSF, diet formulations, method of digestibility measurement, fish size, rearing conditions and culture systems used in studies. Nevertheless, insufficient data prevented us from including these factors in the present analysis, although they might influence greatly the fish response to BSF. Therefore, this topic should be revisited when more research findings are available to identify the various factors affecting the response of salmonids to BSF in diets, which is important for drawing concrete conclusions and making recommendations.

## 5 | CONCLUSIONS

In the present meta-analysis, the full data set showed that, on average, the growth rate, feed conversion, feed intake, protein digestibility and utilization in salmonids fed BSF diets did not differ from those fed control diets. Variations in these parameters, however, existed between the studies. The fish species, type of protein source(s) replaced and BSF inclusion level were partially responsible for variation in fish SGR, whereas only the type of replaced protein source(s) and BSF inclusion level were detected as factors explaining the variations in FCR and feed intake respectively. The meta-analyses of the sub-data sets sorted according to the protein source(s) replaced showed that replacement of fishmeal by BSF decreased SGR and feed intake of salmonids whereas the replacement of non-fishmeal sources improved SGR and feed conversion. This stressed the importance of type of replaced protein source(s) when evaluating the nutritional value of BSF for salmonids. Overall, the present meta-analysis showed that BSF is a promising protein source for salmonid feeds, but its effectiveness is mainly dependent on the type of replaced protein source(s).

## ACKNOWLEDGEMENTS

This work was part of a PhD program funded by SureAqua Nordic Center of Excellence (Grant no. 82342) and the Research Council of Norway (RCN), BioTek 2021/Havbruk Biofeed (Grant no. 229003).

## CONFLICT OF INTEREST

The authors declare no competing conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Weththasinghe P, Hansen JØ, Mydland LT, Øverland M. A systematic meta-analysis based review on black soldier fly (*Hermetia illucens*) as a novel protein source for salmonids. *Rev Aquac*. 2021;00:1-19. <https://doi.org/10.1111/raq.12635>