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Review

Improving the Lipid Profile of Black Soldier Fly (*Hermetia illucens*) Larvae for Marine Aquafeeds: Current State of Knowledge

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Abstract: The replacement of fish meal and fish oil by insect-based ingredients in the formulation of marine aquafeeds can be an important step towards sustainability. To pursue this goal, the modulation of the lipid profile of black soldier fly larvae (*Hermetia illucens*) has received great attention. While its nutritional profile can shift with diet, the ability to modulate its lipidome is yet to be understood. The present work provides an overview of the lipid modulation of *H. illucens* larvae through its diet, aiming to produce a more suitable ingredient for marine aquafeeds. Marine-based substrates significantly improve the lipid profile of *H. illucens* larvae, namely its omega-3 fatty acids profile. An improvement of approximately 40% can be achieved using fish discards. Substantial levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two essential fatty acids for marine fish and shrimp species, were recorded in *H. illucens* larvae fed on fish discards and coffee silverskin with *Schyzochytrium* sp. Unfortunately, these improvements are still deeply connected to marine-based bioresources, some still being too costly for use at an industrial scale (e.g., microalgae). New approaches using solutions from the biotechnology toolbox will be decisive to make *H. illucens* larvae a feasible alternative ingredient for marine aquafeeds without having to rely on marine bioresources.

Keywords: insect feeds; long-chain PUFA; marine aquaculture; *n*-3 fatty acids; highly unsaturated fatty acids; alternative ingredients; fish oil; fish meal

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1. Introduction

Insects provide numerous services and natural products with considerable commercial interest, ranging from feed to biofuel production, waste reduction, textile, pharmaceutical and cosmetical industries, among others [1,2]. In the last decade, the interest in insects as food and feed has experienced its fastest growth [3], despite its nutritional potential having already been acknowledged at least four decades ago [4–6]. More recently, for instance, it was found that coastal flies displayed interesting amounts of polyunsaturated fatty acids (PUFA) [7]. In Europe, this interest mostly arose after July 2017 when the European Union allowed the use of insect meals for aquafeeds formulation [8]. This decision mostly resulted from the urge to find sustainable and nutritious alternative ingredients for aquafeeds [9].

Under this context, several studies using the larvae of *Hermetia illucens*, popularly known as Black Soldier Fly (BSF), forecast its potential as an aquafeed ingredient [10–12]. This species is well-known for its protein level, amino acids profile, lipid fraction, specific fatty acids, and micronutrients content [13,14]. However, its biochemical composition is not always consistent, e.g., crude protein content can range from 40% to 54%, while lipid can vary from 15 up to 49% [15]. Moreover, the lipid fraction per se lacks important essential fatty acids (EFA) for marine fish and shrimp species, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have a crucial role in the growth and health of most farmed marine organisms [16,17]. Under these circumstances, research on the use of *H. illucens* larvae as an ingredient for marine aquafeeds has received more attention concerning the modulation of its lipid fraction [18].

When selecting a raw material destined to be incorporated in the formulation of a diet for aquafeeds, several issues need to be addressed beforehand [19]. One needs to consider the receptor species, along with its life cycle stage and nutritional needs [20]. Concerning raw material, one needs to account for its safety, availability, biochemical profile and stability overproduction, storage, processing, cost, and sustainability [21]. On the production side, it is important to understand if and how it complements other ingredients, if it requires a shift in the production system, and if it changes the final product (aquafeeds floatability, palatability, etc.) [22]. Most aquafeeds currently rely on terrestrial plant sources that must be pre-treated to remove anti-nutritional factors and upgrade the bioavailability of nutrients [23]. Additionally, the nutrient profile concerning amino acids and fatty acids of terrestrial plant sources is not balanced, and the amount of carbohydrates, namely starch, and fiber, may jeopardize the health of farmed marine fish [24]. In that scope, it is urgent to find alternative substrates that are sustainable and low-cost that may provide long-chain PUFA. Genetically modified oilseeds (GMO) can be putative candidates [25,26]. Unfortunately, the costs of GMO seeds, their negative public perception, the need for regulatory approval, and the overexploitation of land and freshwater resources with high environmental and economic costs are some of the multiple drawbacks impairing a more generalized use of these enhanced oilseeds for marine aquafeeds [26,27]. Anti-nutritional factors and industrial processing constraints may be avoided if enhanced oilseeds are supplied as a substrate to *H. illucens* larvae. With no dietary restrictions, *Hermetia* larvae would be able to digest and assimilate the much-wanted omega-3 fatty acids, and the stigma of directly using a GMO in aquafeed could be somehow overcome.

Unicellular sources, such as microalgae or genetically engineered yeasts, are promising choices on what concerns PUFA sources [28,29]. In the case of microalgae, their potential use as sustainable ingredients for aquafeeds has already been documented [28,30]. Nevertheless, several upscaling obstacles must still be overcome to allow microalgae to become a competitive, stable, and affordable bioresource [29]. In the case of genetically engineered yeasts, a life cycle assessment displaying production and environmental costs, along with industry dissemination and upgrading, revealed that these are still constraining their broader use as a source of PUFA [31]. Another subject of interest is that most research efforts seeking alternatives to fish meal (FM) or fish oil (FO) pursue this goal decoupled. This likely occurs mostly due to the specialization of these two different research areas within the field of fish and shrimp nutrition, one more oriented towards protein metabolism, the other more oriented towards lipid metabolism [21]. However, there is a greater potential in merging these two approaches. Finding a raw material able to supply not only essential amino acids but also the much-needed essential fatty acids in a sustainable and low-cost way would be a major advance for the marine aquafeeds industry [3]. In that regard, *H. illucens* larva is well-recognized as a protein provider for animal feed, with a production system that is becoming mature and open to market needs [32]. As cultured marine fish and shrimp are highly demanding species concerning the quantity and quality of fatty acids (FA) present in aquafeeds, *H. illucens* larval biomass with an enhanced fine-tunned lipid profile could be an appealing ingredient [23]. However, to keep *Hermetia* racing alongside microalgae and yeasts, a major and cost-effective enhancement of their PUFA content must be achieved. For that, new approaches concerning genetics, biotechnology, metabolomics, proteomics, and lipidomics must be pursued [24]. Ongoing research targeting the expression of genes involved in the synthesis pathways of fatty acids may enable α-linolenic acid (ALA), EPA, or DHA production on *H. illucens* larvae and place them at the same level of microalgae and yeasts as PUFA providers.

Despite the increasing interest in the use of *H. illucens* larvae as an aquafeed ingredient [9,15,33], a comprehensive review addressing the improvement of its lipid fraction for marine aquafeeds, namely in PUFA, fatty acids ratios, and feeding substrates used to modulate BSF lipid profile, is still missing. The present work provides a systematic overview of key breakthroughs that have allowed the shaping of the lipid pool of *H. illucens* larvae, with emphasis on *n*-3 PUFA, namely EPA and DHA. Our study also aims to provide general guidelines on how the biotechnological toolbox can provide solutions to advance the state of the art on this topic and decrease the dependence on using marine-based bioresources.

2. Materials and Methods

2.1. Literature Search

In order to summarize the current trends on the use of different substrates already tested to feed BSF (objective 1) and the achievements concerning the modulation of its lipid fraction (objective 2), a systematic literature search was performed [34]. All peer-reviewed journal articles were retrieved on January 2022 from the databases Web of Science™ (WoS™) and Scopus using the following keywords and search syntaxes: [“*Hermetia illucens*” AND (“fatty acids” OR “PUFA” OR “*n*-3” OR “omega 3” OR “HUFA” OR (“fatty acids” AND “review”) OR (“*n*-3” AND “review”) OR (“omega 3” AND “review”))] and [“Black Soldier Fly” AND (“fatty acids” OR “PUFA” OR “*n*-3” OR “omega 3” OR “HUFA” OR (“fatty acids” AND “review”) OR (“*n*-3” AND “review”) OR (“omega 3” AND “review”))]. The search options selected in Scopus were article title, abstract, and keywords, with no date restriction (Table 1). In WoS™, the keywords and syntaxes were searched for in the “topic” selection, with date limitation from 1900 to 2020 (Table 1). Using all the keywords and search syntaxes, a total of 546 publications were retrieved from WoS™ and 581 from Scopus. Duplicate titles were removed from the pool of retrieved publications. The subsequent material collected from WoS™ and Scopus totaled 392 publications. These outcomes were then categorized by author, title, year, journal, and DOI.

Table 1. Results delivered by Web of Science and Scopus addressing the listed keywords.

Keywords	Web of Science™	Scopus
Hermetia illucens + fatty acids	196	214
Black Soldier Fly + fatty acids	183	205
PUFA + *Hermetia illucens*	23	21
PUFA+ Black Soldier Fly	23	24
n-3 + *Hermetia illucens*	32	34
n-3 + Black Soldier Fly	27	33
omega 3 + *Hermetia illucens*	8	10
omega 3 + Black Soldier Fly	23	26
HUFA + *Hermetia illucens*	2	2
HUFA + Black soldier fly	2	2
Hermetia illucens + fatty acids + review	18	6
Black Soldier Fly + fatty acids + review	7	2
PUFA + *Hermetia illucens* + review	0	0
n-3 + *Hermetia illucens* + review	1	0
omega 3 + *Hermetia illucens* + review	1	2
HUFA + *Hermetia illucens*+ review	0	0
Total of publications	546	581
Total of publications considered after removing duplicates	392	
Total of publications considered after selection criteria	47	

2.2. Inclusion Criteria and Data Extraction

Further selection procedures combined two steps: the analysis of: (a) title, (b) abstract, and (c) results and a search for the following benchmarks: (1) BSF larvae feeding tests, (2) fatty acid analysis, and (3) detailed description of fatty acid content. Each publication was screened individually to determine whether it was considered or discarded for the present study. The final number of relevant publications selected was 47 (Table 1).

From these results, we built a table (Supporting Information Table S1) compiling the relative abundance of molecular species allocated to the lipid fraction identified in the larvae of *H. illucens* fed with the substrates described in each publication selected. A total of 148 substrates were identified, and 46 molecular species of fatty acids were retrieved; these were subsequently divided into Saturated Fatty Acids (SFA) (20 species), Monounsaturated Fatty Acids (MUFA) (12 species), and Polyunsaturated Fatty Acids (14 species). For further analysis, the substrates were categorized into nine groups: Control (chicken feed), Cereal, Fruit, Manure, Seafood, Seaweeds, Vegetables, Waste, and Miscellaneous (Supporting Information Table S2). When the substrate described corresponded to a mix encompassing more than one of the categories considered it was included in the group with the highest percentage of inclusion, e.g., chicken feed + 4% of flaxseed oil—considered as control group (chicken feed). However, in the case of seafood and seaweeds every substrate containing a source of this category regardless of the percentage employed was included in these categories, e.g., 10% fish offal (rainbow trout) and 90% cow manure—considered as seafood.

2.3. Statistical Analysis

After the critical survey of the scientific literature retrieved, we performed a systematization and analysis of all data gathered on the fatty acid profile of larvae fed with the different substrates described in the publications. The dataset was analyzed using a chemometric statistical method aiming to obtain a biologically relevant perspective by looking into patterns in lipid molecular species within the groups of feeding substrates considered. Statistical analysis was performed using MetaboAnalyst (v5.0) [35,36]. Prior to analysis, the percentage of relative abundance of each lipid species of the larvae of *H. illucens* fed with the substrates considered for this survey was collected. Aiming to forecast the correlation between substrates and the amount of *n*-3 fatty acids in larval tissues, only ALA, EPA, DHA, PUFA, and SFA were considered for statistical analysis (Supporting Information Table S2). Feeding-substrates provided to BSF were categorized in nine groups as described above.

Data normalization, namely log-transformation followed by auto-scaling, was performed prior to analysis to decrease the influence of molecular species with high-abundance and increase the statistical relevance of the ones present at lower abundances (Supporting Information Figure S1). A Principal Components Analysis (PCA) was performed to highlight the differences in lipid molecular species displayed by BSF larvae provided with each group of substrates. A hierarchical cluster analysis was also performed using the Euclidean distance similarity measure, as well as a Ward's linkage visualized in a heatmap and a dendrogram.

3. Results & Discussion

3.1. General Framework

After data categorization by year (Figure 1), it became clear that 2017 was the turning point in the research field being covered in the present study. With this survey, it was evidenced that publications addressing the lipid content of *H. illucens* larvae have grown exponentially in recent years, showing the timely need for the present review.

To address the different scientific fields covered in the literature retrieved, publications were grouped by journal (Supporting Information Table S3). *Aquaculture*, *Animals*, and *Journal of Insects as Food and Feed* were the top three peer-reviewed scientific journals publishing on *H. illucens* and its lipid profiles. These findings suggest that the study of *H. illucens* larvae as a feed ingredient for aquaculture has gained momentum, with the research community acknowledging the need to tailor the biochemical profile of this emerging ingredient for aquafeeds. Modern-day aquafeed formulation is well beyond the mix of single amounts of each ingredient to fulfil a balanced pool of nutrients to the target species being cultured. In fact, it rather targets the balanced-dosage of multiple ingredients, aiming to cover the nutritional needs of a given species at a specific life cycle stage during production [21]. *Hermetia illucens* larva is a good candidate as an aquafeed ingredient, meeting multiple criteria for such purpose i.e., a suitable biochemical profile, well-established industrial processing and already having been validated as an ingredient in different formulations for several species, including marine organisms. Additionally, the ability of *H. illucens* larvae to bio-convert wastes and industrial side streams became a popular feature, namely when advocating circular bioeconomy approaches that have positive environmental impacts [37,38]. The use of *Hermetia* larvae for this end allows to close nutrient cycles, reduce problematic wastes, and give origin to a highly versatile, nutritious feed substrate that can be produced under controlled conditions and scaled-up to meet demand [13]. Moreover, the high percentage of crude lipid in the larvae of BSF led to significant research on methods to attain better extraction yields and an increase in fat [37,39–43].

Figure 1. Number of peer-reviewed publications retrieved from Web of Science and Scopus concerning *Hermetia illucens* and fatty acid related keywords (after removing duplicates).

To date, multiple studies have documented the use of different feeding substrates to produce *Hermetia* larvae framed within variable goals, from the bioconversion of wastes [44–48], to the production of biodiesel [37,39–43], or the modulation of the nutritional profile of *H. illucens* [33,49–51].

It is well documented that *H. illucens* larva is able to feed on a multitude of substrates, including manure, fish offal [33], brewery and winery by-products [38], restaurant waste [52], as well as fruits or vegetables [33,38,52,53]. In this survey, cereal, manure, fish, and seafood were the top three substrates used to feed *H. illucens* larvae (Figure 2). These findings highlight that, in recent years, multiple studies have been pursuing the application of *H. illucens* larvae as an ingredient in aquafeeds, aiming to enhance its levels of FA unsaturation and long chain *n*-3 PUFA.

3.2. Lipid Profile of *Hermetia illucens* Larvae-From Quantitative to Qualitative Approaches

Initially, insects gained the interest of feed producers due to their protein content [54,55]. Later it was found that insect oils were a valuable resource for animal nutrition, as they displayed exceptional properties and a lipid content comparable to plant oils [55]. The amounts of SFA and unsaturated fatty acids displayed by *Hermetia* larvae are similar to those of sunflower or cottonseed oils, already used in the feed industry [56]. Moreover, insect oils were demonstrated to have considerable levels of sterols, a group of biomolecules known to be highly stable [56]. Additionally, by having a low content in PUFA, insect oils display high oxidative stability, a feature that is not commonly displayed by other oils [57]. In general, the lipid content of insects does not display long-chain fatty acids longer than C18:3 [58]. Additionally, their biochemical profile is known to be species-specific and dependent on life cycle stage, feeding substrate, and several environmental parameters, such as temperature, humidity, and light [59]. Studies on the lipid fraction of *H. illucens* larvae emerged fostered by the quest to find sustainable alternative sources to produce biodiesel, which would not compete with other substrates already used as human foods. In that regard, a large amount of literature described methods and compared the performance of supplying different feeding substrate aiming to enhance the lipid content of *H. illucens* larvae. For instance, Abduh et al. (2017) [60], demonstrated that the oil content of *H. illucens* larvae varied from 19 to 28 weight percent (wt%) depending on whether or not rubber seeds were pre-treated with a mix of microorganisms. In a study by Zheng et al. (2012) [61], the lipid content of *H. illucens* larvae reached 39 wt% using rice straw as feeding substrate, with this displaying a high content of cellulose, hemicellulose, and lignin, a mix of microbes (Rid-X) and restaurant waste. These studies provided substantial data on the lipid content of *H. illucens* larvae fed under different substrates and allowed a better understanding of qualitative lipid modulation in this insect species. Currently, the use of insects for marine aquafeeds opened a whole new set of questions related with their lipid profile. The major concern for marine aquafeeds formulation refers to the quality, rather than the content, of lipids, more precisely, the molecular species that are available in the lipid pool, namely PUFA and highly unsaturated fatty acids (HUFA) (FAs with 4 or more double bonds on their carbon chains). In that regard, St-Hillaire et al. (2007) [33] pioneered the studies that aimed to modulate the lipid profile of *H. illucens* larvae for aquafeeds by using trout fish offal as a substrate. This approach aimed to improve the ratio of ALA, EPA, and DHA in aquafeeds being supplied to fish. In fact, the amount of *n*-3 fatty acids went from negligible to nearly 3% of the total pool of FA present in the biomass of larval *Hermetia*, even in short-term tests (24 h). Nevertheless, despite the levels of ALA, EPA, and DHA being below the needs of most marine species, the results achieved opened good perspectives for future breakthroughs in this research field.

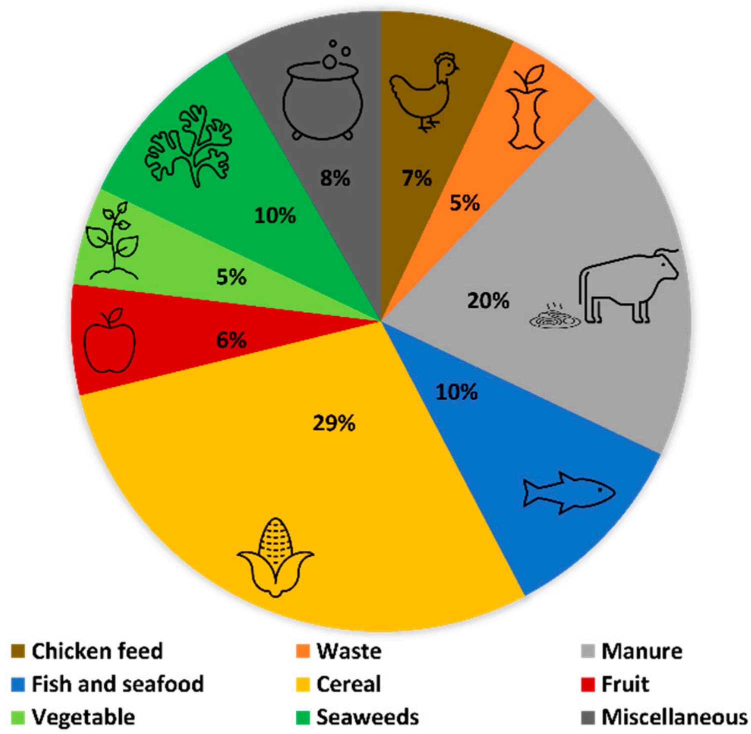

Figure 2. Top 10 substrates used to feed *Hermetia illucens* larvae according to publications retrieved from Web of Science and Scopus concerning *Hermetia illucens* larvae and their fatty acids profile.

3.3. Manipulation of the Lipid Content of Hermetia illucens for Marine Aquafeeds Formulation

The use of insects as an ingredient for aquafeeds and as a potential substitute for FM is now widely acknowledged [23,62]. The natural amino acid profile and the protein content of *H. illucens* larvae is suited for most aquafeeds and continues to gain growing attention [63]. In the case of the lipid content, the high levels of SFA, along with the low content of PUFA, jeopardize the direct use of *Hermetia* as a reliable substitute for FO when it comes to marine fish or shrimp. As demonstrated in this review, several studies already tried, and succeeded, to promote an increase in the PUFA content of *Hermetia* larvae, namely on ALA, EPA, and DHA. Recent developments show that feeding substrates that gave origin to *Hermetia* larvae with the highest contents in *n*-3 fatty acids were fish discards (39.9%), coffee silverskin supplemented with *Schyzochytrium* sp. (28.5%), and seaweeds (14.1%). Improved *n*-3/*n*-6 ratios were achieved by Ewald et al. (2020) [17] with fish, while Spranghers et al. (2017) [52] achieved that goal with vegetable waste, and Truzzi et al. (2020) [50] with coffee silverskin + 5% *Isochrysis* sp. The content of essential FAs for marine fish and shrimp (such as EPA, and DHA) in *H. illucens* larvae was related to the type of substrates used during larval feeding. In the case of ALA, the most significant levels were obtained using chicken feed with a supplementation of 4% of flaxseed (9.7%) [64], and vegetables (5.8%) [53]. Relevant levels of EPA and DHA were recorded in *H. illucens* larvae supplied with fish discards (13.6% and 21.4%, respectively) [49], as well as when supplied with coffee silverskin with *Schyzochytrium* sp. (11% and 16%, respectively) [50].

For the present review, 148 fatty acid profiles of *H. illucens* larvae (Table S1) were allocated to 148 different feeding substrates. The average lipid profile assembled from this search (Figure 3) displays five major contributors for SFA: lauric acid (C12:0), palmitic acid (C16:0), myristic acid (C14:0), stearic acid (C18:0), and arachidic acid (C20:0). Lauric acid is the most abundant fatty acid found in larvae of *H. illucens*. According to Spranghers et al. (2017) [52], the abundance of this fatty acid is not conditioned by the stage of development of *H. illucens*. When analyzing the data retrieved during the present work (Figure 3 and Table S1), this dichotomy is clearly associated with the feeding substrate provided. Feed with a marine origin, such as seaweeds, fish, or mussels present zero or very low values of this fatty acid. The highest values are found in feeding substrates using fruits, vegetables, and some cereals.

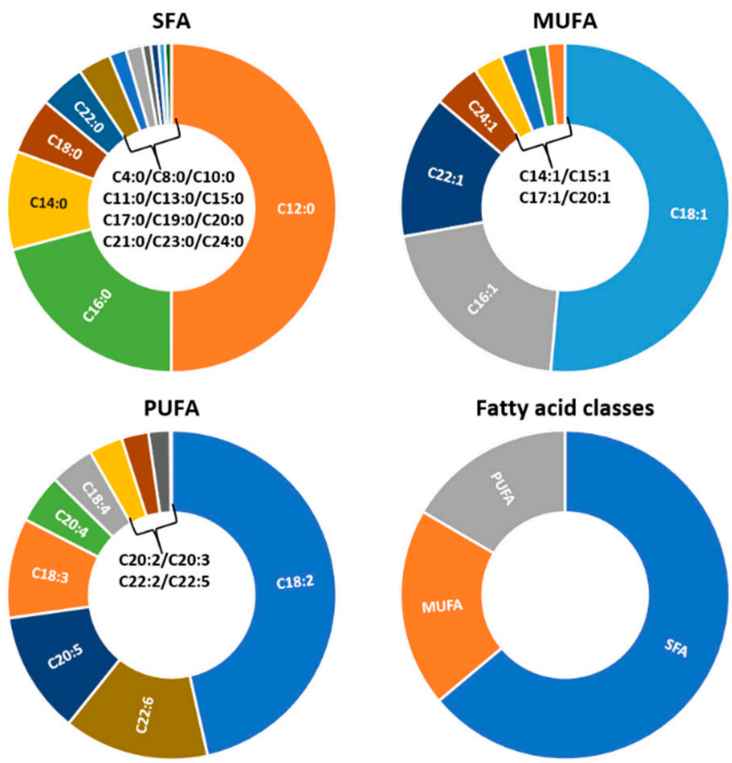

Figure 3. Average lipid profile of *Hermetia illucens* larvae obtained from analysis of 148 lipidic profiles of larvae fed with 148 substrates. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

In the case of MUFA (Figure 3) it is possible to distinguish four great fatty acids: vaccenic (C18:1 (*n*-7)) and oleic acid (C18:1 (*n*-9)), grouped as C18:1, palmitoleic acid (C16:1 (*n*-7)) and 9-*cis*-Hexadecenoic acid (C16:1 (*n*-9)), grouped as C16:1, erucic acid (C22:1 (*n*-9)) (C22:1 (*n*-1)) and nervonic acid (C24:1 (*n*-9)). Oleic acid is the most common fatty acid in nature, and it was found in all data analyzed in this survey. Results ranged from negligible amounts to a maximum of 32% when *Hermetia* was supplied with cow manure [33,65]. As part of the fatty acids that constitute fat tissue, palmitoleic acid is widely common and found in most study cases of this review, with the exception of *H. illucens* larvae fed with Philippine tung seed [66], cow manure, cow manure with fish, and pig manure [33,65]. Despite its wide availability, the maximum value registered was 10% [53]. In the case of 9-*cis*-hexadecenoic acid, the main results exhibiting this fatty acid ranged from 6.1% with rye meal [56] to 0.006% with cornmeal and fruit and vegetable mixtures (50:50) [67]. With few examples found in the literature in this scope, erucic acid was found in its *n*-1 form in fish discard with 2.4% [49], and in *n*-9 in layer mash 0.08% and fish offal layer mash with 0.03% [68]. The maximum level was observed in coconut endosperm and soybean crude residue (3:2) at 7.28% [69]. Nervonic acid was detected on *H. illucens* larvae in lower levels, being mostly present when *Hermetia* larvae were fed fish discards [49] or chicken feed [70].

Further analysis of PUFA results show four fatty acids with a major contribution: linoleic acid (C18:2 (*n*-6)), α-linoleic acid (C18:2 (*n*-3)) (presented as C18:2), DHA (C22:6), EPA (C20:5), α-linolenic acid (C18:3 (*n*-3)), and γ-linolenic acid (C18:3 (*n*-6)). Linoleic acid is one of the most common PUFAs in insects. The *n*-6 conformation was found in most studies retrieved, with only a few exceptions, such as when feeding *Hermetia* larvae with palm kernel meal [71], ice cream industrial waste [71], coconut endosperm and soybean crude residue (3:2) [69], and food waste [72]. The percentages registered ranged from 0.49% [73] to 52.5% [49]. The *n*-3 form was found exclusively when using pig manure at 2%, dog food, and human faeces (4:4:2) [74]. Consistently, DHA was detected in *H. illucens* larvae reared on marine sources [17,33,49,50,75–78], with fish discards being the substrate that yielded the highest levels of this fatty acid (21.4%) [49]. EPA presented the same outline, with marine sources being the privileged source for this fatty acid [16,17,33,50,68,75–81], again with fish discards as its major contributor (13.6%) [49]. However, in minor amounts, other substrates (food waste [17], pig manure [65], soybean crude residues [82] and others [32,51,74,82]) also evidenced this fatty acid in *Hermetia* larvae. Considering α-linolenic acid (C18:3 (*n*-3)) and γ-linolenic acid (C18:3 (*n*-6)), the *n*-3 form was the dominant conformation detected in most substrates in levels going from 0.19% [68] up to 9.7% [64]. This fatty acid is of major relevance due to its ability to be bio-converted into highly unsaturated fatty acids by some marine and freshwater fish species. The *n*-6 conformation was also found, although in lower amounts (0.02% [68] to 2.6% [53]) and in fewer substrates [50,65,68,83].

When analyzing the proportion of the three classes of fatty acids considered in this work, SFA represented 60% of the of total of fatty acids. This is the most common class of fatty acids found in insects, with *H. illucens* larvae being no exception. Major levels of SFA were found in larvae reared on commercial broiler chicken feed (93.9%) [84], coconut endosperm (87%) [69], and fruit (86%) [53]. The average values of MUFA retrieved in this review fluctuated around 20%. The maximum values recorded where those for *H. illucens* larvae fed with seaweed and coconut endosperm (34.4%) [69,79], ensile mussels [17], and livestock manure (32.1%) [65]. PUFA represented nearly 20% of the pool of fatty acids of *H. illucens*. Higher levels of these fatty acids were found when using Philippine tung seed (67.6%) [66], a control diet employed by Barroso et al. (2019) (57.0%), and coffee silverskin with 25% of *Schyzochytrium* sp. (37.8%) [17]. It is worth highlighting that C18 chain fatty acids were the dominant source for these results.

From the principal component analysis, we found five principal components, each one explaining the following percentage of total variance: PC1 43.5%, PC2 25.9%, PC3 19.3%, PC4 8.2% and PC5 3.1%. The first two axes together accounted for 69.4% of total variance (PC1 43.5%; PC2 25.9%) (Figure 4). The variables associated to PC1 and PC2 are substrate types (manure, cereals, seafood, etc.) vs. fatty acids (SFA, PUFA, ALA, EPA, and DHA).

PC1 was dominated by ALA, EPA, and DHA, with *H. illucens* larvae reared on seafood and seaweeds showing a higher percentage of these FA and being easily differentiated in the data cloud. PC2 was dominated by the contrast between SFA (negative scores) and PUFA (positive scores).

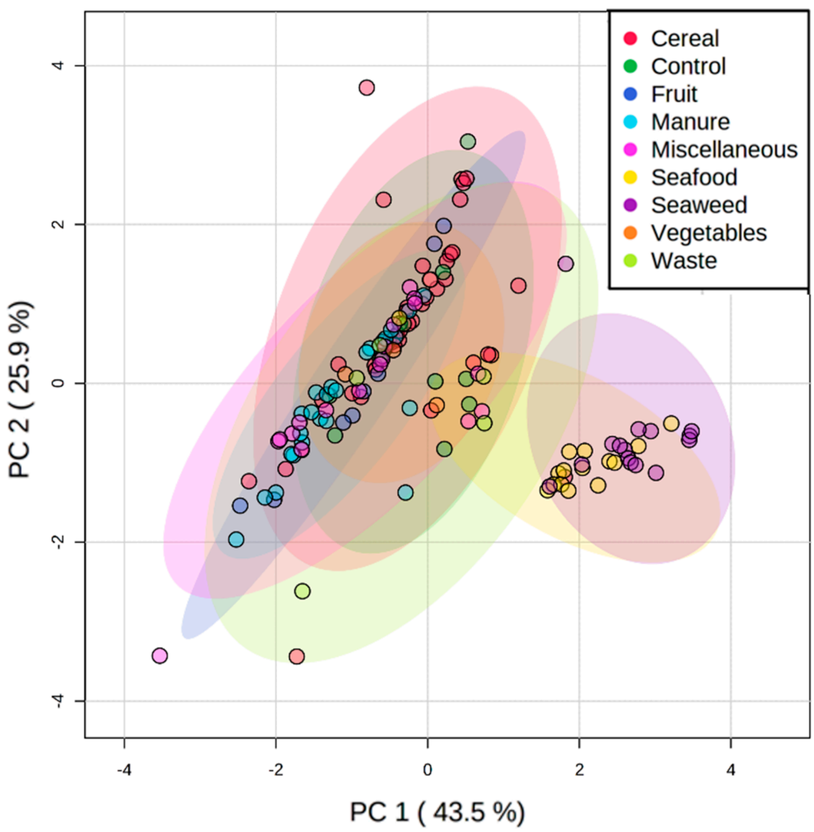

Figure 4. Score plot of Principal Components Analysis (PCA) of fatty acid profiles of *Hermetia illucens* larvae fed on different substrates (*n* = 148) displaying the first two Principal Components (PC) (PC1 and PC2) of the five-axis identified. The variance explained by PC1 and PC2 is shown between brackets.

The differences among the FA profiles of larvae supplied different feeding substrates are represented on the heatmap and dendrogram in Figures 5 and S2. These results emphasized the presence of DHA and EPA in seaweeds and seafood and separate fruit and manure as substrates with low levels of ALA, EPA, DHA, and PUFA. Furthermore, cereals and fruits presented the lowest levels of SFA and PUFA, respectively. The dendrogram isolated two major groups, with seaweeds and seafood being separated from all substrates surveyed. Moreover, SFA is separated as well from the other four groups, which were clustered in pairs: DHA with EPA and ALA with PUFA.

The main finding of the present analysis is that *H. illucens* larvae fed with marine-based substrates display an improved lipid profile, showing higher levels of EPA, DHA, or PUFA. Thus, whenever possible, marine-based substrates should be favored when culturing *H. illucens* biomass destined to be used for aquafeeds being formulated for marine fish and shrimp, thus targeting higher levels of nutritionally relevant PUFA and HUFA. Nonetheless, it is worth highlighting that this dependence on marine-based substrates may by itself be a potential bottleneck. However, such substrates do not need to undergo any further processing and may be directly provided to *H. illucens* to obtain an enriched larva on *n*-3 PUFA and HUFA. This is not the case when these same marine-based substrates (e.g., fish offal) are used to formulate aquafeeds for marine fish/shrimp [21]. This approach may allow the reduction of production costs of marine aquafeeds, making it possible to incorporate in a single ingredient balanced protein and lipid contents.

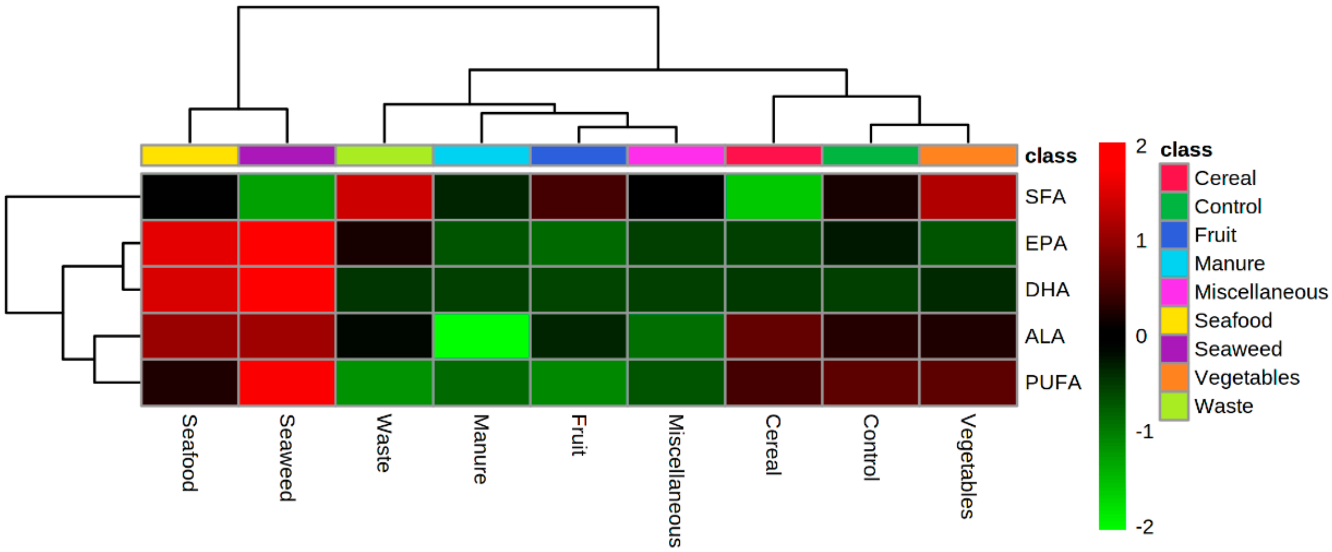

Figure 5. Heat map of fatty acid profiles of *Hermetia illucens* larvae fed on different substrates (n = 148) using fatty acid relative abundances (log-transformed and auto-scaled) and ranging from down-accumulated (green) to up-accumulated (red). Hierarchical clustering was based on Euclidean distance similarity measure and Ward's linkage.

4. Conclusions and New Research Pathways

There are clear benefits associated with the mass production of insects using approaches that do not compete with food production for human use, which enhance waste management and nutrient recycling [85]. However, there are still some technological constraints concerning the mass-rearing of insects that need to be overcome. Up-scaling strategies allowing continuous production with consistent quantities and quality need to be implemented, while reducing production costs and making insect meals more competitive [86]. Nevertheless, the growing development of mass-rearing systems, along with the forecasted economic crisis and/or the increase of food and feed prices, sets the stage to further explore creative solutions to these current bottlenecks. An in-depth understanding of *Hermetia illucens* larval lipid metabolism and lipidome, if supported by trials testing its metabolic plasticity and environmental limits, are key to benefit the most from feeding substrates currently available to mass produce *H. illucens*. Moreover, biotechnological approaches should be considered to improve the nutritional bioavailability of substrates and upraise the yields of dietary incorporation, namely for polyunsaturated fatty acids (PUFA). Research exploring the potential existence of dormant metabolic pathways on *H. illucens* larvae or its associated gut microbiome, that may be triggered to allow the production of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from α-linolenic acid (ALA) precursors must also be pursued. Published results on the modulation of the fatty acid profile of *H. illucens* larvae here analyzed are certainly promising but, with a few exceptions, are still unable to fulfil the nutritional needs of marine fish and shrimp species currently being farmed. With a biomass rich in essential amino acids and minerals, it is most certain that in upcoming years *Hermetia* larvae will become a regular ingredient in aquafeeds formulations. Its use will certainly increase for premium batches of this ingredient featuring high levels of essential n-3 PUFA and highly unsaturated fatty acids. The present review aims to inspire researchers to pursue innovative approaches that may allow the unleashing of the true potential of *H. illucens* as a key ingredient for the formulation of marine aquafeeds, supporting a more sustainable aquaculture and contributing to the harnessing of Blue Foods.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su14116472/s1, Table S1: Relative abundance of molecular species of fatty acids (expressed as % of total pool of fatty acids) of *Hermetia illucens* larvae fed with different substrates retrieved from the literature surveyed; Table S2: Dataset used on MetaboAnalist to perform the statistical analysis; Table S3: Top 10 peer-reviewed scientific journals publishing scientific research addressing the fatty acid profile of *Hermetia illucens* retrieved from WoS™ and Scopus. (Journals publishing 5 or less articles on this topic were grouped as Others); Figure S1: Box plots and kernel density plots before and after normalization. The boxplots show at most 50 features due to space limitations. The density plots are based on all samples. Data transformation: Log Normalization; Data scaling: Autoscaling; Figure S2: Hierarchical clustering of substrates shown as dendrogram (distance measures using Euclidean, and clustering algorithm using Ward's Distance).

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