Literature review to define knowledge gaps on the sustainable production of insects for feed

This literature search was written in context of the Interreg North-West Europe ValuSect project (NEW1004).

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Introduction

This literature search is an addition to the literature review performed for work package one within the ValuSect project, in which different topics regarding the sustainable production of insects for food are considered. In the literature search of WP1, highly relevant knowledge was obtained in order to be able to conduct applicable and realistic experiments, to clarify questions from SMEs and companies regarding the sustainable production of insects, and to include the production of insects on side streams and the measurement of insect emissions. However, this literature search was limited to three insect species with potential in human food applications, namely the yellow mealworm (*Tenebrio molitor*), the house cricket (*Acheta domesticus*) and the migratory locust (*Locusta migratoria*). Meanwhile, the ValuSect project has been further extended for the inclusion of insect production for the animal feed industry and black soldier fly (*Hermetia illucens*) as fourth insect species.

The black soldier fly has gained increased attention recently due to its potential in many applications. For example, mass production of black soldier fly can result in several products such as protein (meal) and oil. This literature search focusses on the production of black soldier fly for animal feed applications, referring to the scope of the ValuSect project. The black soldier fly (BSF) (*Hermetia illucens*) is considered to have huge potential in animal feed applications due to some interesting properties of this insect species. For example, black soldier fly larvae can be produced with a lower environmental impact than conventional protein sources. They are able to consume organic waste and efficiently convert this low-value biomass into high-value components, making them a rich source of proteins. Therefore, black soldier fly larvae can possibly become a more sustainable protein source for animal feed than current sources. This insect species might be able to close the loop, making them a good fit within the circular economy strategy. However, before insects in general can become applicable as sustainable protein source, the production process needs to be optimal from an economical and productivity point of view. As the industrial production of insects for agricultural applications is a relatively new concept in Western countries, this is not yet the case.

The aim of this literature search is to define knowledge gaps on the sustainable production of insects used in animal feed. The focus is on the BSF insect, as this species is considered the most promising for animal feed applications. The knowledge here obtained will be used to plan and conduct relevant experiments aiming to fill the identified gaps and to contribute to the optimisation of the insect production process.

This literature search is carried out as part of the Interreg NWE ValuSect project (work package 5: insects for feed, activity 1: transferring knowledge build on insects for food to insects for feed), in which all project partners of work package 5, Activity 1 are actively involved. The aim of this literature search is to define knowledge gaps on the production of insects for feed applications.

The ValuSect project initially focused on 3 insect species suited for human food, namely Tenebrio molitor (yellow mealworm), Acheta domesticus (house cricket) and Locusta migratoria (migratory locust). During the ValuSect project, a call for capitalization regarding insects for feed has been submitted and approved, thereby including the black soldier fly (Hermetia illucens).

The objective of the ValuSect project is to enhance innovation performance of NWE agrofood companies by transferring knowledge on efficient use of insects and insect components as building blocks for new food and feed products. Goals of the project are (1) establishment of a transnational Accelerator program to support enterprises in realising new to the firm/ new to the market solutions and to transfer knowledge, (2) the quality improvement of insect production processes and (3) the quality improvement of insect processing procedure.

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List of abbreviations

ADF	Acid detergent fiber
BSF(L)	Black soldier fly (larvae)
C/N ratio	Carbon nitrogen ratio
ECI	Efficiency of conversion of ingested food
FCR	Feed conversion ratio
U	Functional unit
NDF	Neutral detergent fiber
P:C	Protein carbohydrate ratio
VOC	Volatile organic compound

Clarifying the issue

The global population is expected to reach 9.7 billion people by 2050 [1]. Associated with this continuous growth, as well as the rising prosperity, is the demand for food, which is estimated to increase by 70–80% between 2012 and 2050 [2,3]. In addition, high amounts of biomass, related to the production and consumption of food, is lost [4]. Current food production has a profound impact on climate change and depletion of natural resources. For example, conventional livestock farming is responsible for about 15% of the total greenhouse gas emissions caused by human related activities [2,5]. Furthermore, soybean and fish meal, which can be extensively used as protein supplementation in some animal production systems, are also associated with a scarcity of resources to being produced, in order to satisfy the increasing demand that is leading to a decreased land availability, deforestation and overfishing. This not only puts pressure on the environment, but also on the economy as the prices of common feed ingredients are continuously increasing. As a result, the demand for alternative and more sustainable protein sources is rising [6–8]. Black soldier fly have equivalent protein contents to soybean meal and more crude fat than either fishmeal or soy, making them an excellent choice of feedstock [9].

Insects have the potential to alleviate the above-described societal challenges, and therefore, could be an attractive key player to the circular economy. Insects, generally, need less water leading to a lower water footprint and they can be produced in vertical farming systems, requiring less space compared to current animal protein sources [10–14]. On top of this, it has been reported that when insects are used for food purposes less energy and, therefore, less CO_2 equivalents are generated by kg of digestible material produced [15]. A big advantage of black soldier fly larvae is that they are capable of converting biomass efficiently to body mass, with lower FCR (feed conversion rate) than current livestock [16,17]. Moreover, black soldier fly larvae are reported as feeding on a broad range of substrates including wastes, leading to the potential of valorising side streams, at the same time that waste volumes are reduced. Furthermore, black soldier fly larvae require less time to complete their lifecycle, i.e. 2-3 weeks from egg hatching till harvestable larvae. Besides their high efficiency, insects also contain interesting nutritional aspects even when reared on low-value biomass, making them a rich source of proteins [18,19]. In addition, the black soldier fly insect is not considered a pest species as they do not have stinging or chewing mouthparts as an adult. Therefore, they do not approach humans, do not bite or sting, and do not transmit diseases by their mouthparts. Moreover, the lack of resilience of this insect species to lower temperatures prevents its invasion of cold and temperate regions (e.g. Northern Europe) [18,20–22].

Due to the above mentioned properties, the black soldier fly is considered a promising alternative feed ingredient feed alternative. However, the industrial production of insects is a relatively new concept in Western countries. Insects have been bred on a small scale for some time now, mainly as feed for hobby animals, but their application in livestock feed is virtually absent. This innovative character poses some challenges for the sector. For instance, the need for optimization and automation of the production process. Despite the fact that a lot of research has been done on insect breeding recently, this concept is still in its infancy. After all, most agricultural sectors can rely on 100’s years of practical knowledge and experience, which has led to the high production efficiency of these sectors today. Insect producers have to invest in automation, optimisation and upscaling of the processes in order to reduce costs and increase efficiency, to be competitive when compared to conventional feed products. In addition, insect protein is relatively expensive compared to current protein sources used in animal feeds, such as soy. Examples of methodologies by which insects can be produced more cost-effectively include the use of non-valorised side streams as insect feed, scaling up the processes and limiting manual operations [23–26].

Since the industrial production of insects is a relatively new concept in our Western society, there is a lack of knowledge and data on several topics and further optimizations of sustainable and upscalable production processes are needed. The objective of this literature search is defining knowledge gaps on the sustainable production of black soldier fly and obtain relevant information that will enable to fill these gaps.

Black soldier fly diets

The development of optimal insect diets has been identified as one of the major challenges for the insect production industry. Often, standard diets are used to feed the insects, however these are not specifically designed for the insects. For example, typically chicken meal is used as the diet for black soldier fly, or wheat bran for mealworms. These diets mostly do contain all necessary nutrients for the insects, but often not in optimal proportions. As a result, these diets lead to inefficient production, and possibly increased production costs [25,27,28].

One of the benefits of black soldier fly larvae is that they can efficiently convert low-value biomass into body mass consisting of high-value components such as proteins, fat and chitin. Furthermore, they are reported to be able to thrive on many different substrates, including low-value side streams or wastes [16,17,19]. This is likely to be due to the diversity of digestive enzymes in the gut of BSF compared with other insects, such as the common house fly [29].

The diet is crucial for the economics of insect production. Life cycle analyses (LCA) indicate that the production of black soldier fly larvae can be done more efficiently and cost-effectively if the larvae are reared on low-value biomass such as manure or wastes. In addition, rearing insects on low- and non-valorised organic streams would greatly lower their environmental footprint and increase the sustainability of the production process [6,18,23,26,30]. Consequently, interest in the production of black soldier fly larvae on side streams has increased over the recent years, resulting in many studies being generated [18,23,31,32]. As this topic has been widely explored, the focus of this literature search will be delineated to diets with the most potential for black soldier fly mass rearing. The focus will be on diets performing equally or better than standard diets in terms of larval growth and survival, end weight and conversion (if mentioned). Before optimal diets can be defined, the dietary requirements of black soldier fly larvae must be well understood, a topic also addressed in this chapter.

Black soldier fly dietary requirements

Physical requirements

The substrate in which BSF larvae live is of major importance in order to optimise growth. Not only do nutritional factors come into play, but several abiotic "physical" parameters can cause a substrate to be either hospitable or hostile for the larvae. These factors are hard to isolate as their influence is often interwoven with one another. Some of these factors, but not limited to, are;

- Particle size:
  Particle size of the feeding substrate is shown to affect larvae growth. Palma et al. [33] tested almond hulls milled to different sizes and reported that the coarsest milled almond hulls (passing 6.35 mm) yielded larger larvae than larvae reared on the finer milled hulls (passing 4 mm).

- Substrate depth:

Substrate depth was tested by Brits [34] and he concluded that an optimal substrate depth was better not above 10 cm.

- Moisture content:
  As reported by Holmes et al. [35] and Palma et al. [36] increasing BSF diets humidity up to 70 % is beneficial for larval growth. However, the presence of excessive moisture (liquid), negatively affects larvae [37].

- Aeration rate:
  Aeration rate of the substrate will determine how much oxygen is available to the larvae. Passive oxygen exchange will be influenced by substrate depth, particle size (which might influence macro and micro pores), and humidity of the substrate (and associated water saturation of the pore structures). Improved aeration is shown to be beneficial for larvae growth as is shown by Palma et al. [36], they showed that mechanical aeration of the substrate improved larval yield fivefold for their specific rearing setup.

- pH:
  Results from both Popa & Green [38] and Alattar [39] showed that BSF larvae were able to regulate pH of the substrate to a value to almost 9.0 with larval activity. Results from Alattar showed no survival of larvae on a substrate with pH 0.7. Consequently, it was concluded that black soldier fly larvae are not able to thrive on a substrate pH below 1.7 [39]. In the study of Ma et al. [40] different initial substrate pH values were investigated (2.0, 4.0, 6.0, 7.0, 8.0 and 10.0) for effect on growth performances of BSF larvae. pH values of 6.0 – 10.0 resulted in heavier final BSF pupal weights than lower pH values investigated. Larval activity increased pH values of the final substrate to 8.0-8.5, except for the lower initial pH values 2.0 and 4.0, which showed a final pH value of 6.0 [40]. In the research of Meneguz et al. [41] larval growth and development was examined on Gainesville diet (50 % wheat bran, 30 % alfalfa meal, 20 % corn meal) with pH 4.0, 6.1 (control), 7.5 and 9.5. In their study pH influenced larval weight at the beginning of the experiment: substrates with pH 4.0 had the lowest larval weight at the start of the experiment. However, larval activity increased the pH during the experiment and subsequently, no significant differences in larval final weight was found between different treatments [41]. It is important to note that abiotic conditions, such as pH, have an impact on the microbiota of the substrate. As microbial communities have an effect on organisms (e.g. interactions) (see 0), shifts in pH might indirectly impact BSF larvae [41].

Nutritional requirements

Proteins, carbohydrates and their ratio are among the most important nutrients to support insect growth and development [42–46]. In the study of Barragan-Fonseca et al. [47] nine chicken-feed based diets varying in their protein carbohydrate ratio were formulated, ranging from 45–79 % protein and carbohydrate content. Three protein concentrations (10 %, 17 % and 24 %) and three carbohydrate concentrations (35 %, 45 % and 55 %) and their combinations were included in the study.
Survival of the larvae did not seem to be affected by the different treatments. This was expected by the authors as they used diets containing macronutrient contents and ratios similar to those found for nutrient sources consumed by the black soldier fly larvae in nature. The results confirm that the black soldier fly larvae are able to thrive on a broad range of macronutrient ratios.

On the other hand, development time and larval yield were influenced by the different treatments. However, it seems this was more affected by protein and carbohydrate content rather than the protein carbohydrate ratio. Low protein and carbohydrate contents resulted in a shorter development time. Larval yield showed a significant linear effect of carbohydrate content: larval yield was highest on the treatment with the highest carbohydrate content for all three tested protein contents. Larval yield decreased as the dietary carbohydrate concentration decreased. The authors concluded that a high macronutrient content combined with a low protein carbohydrate ratio positively affects *H. illucens* performance.

A review paper from De Smet et al. [48] includes the variety of substrates that have been tested for BSF rearing. Although the larvae were able to grow on many diets discussed, the length of time required for the insect to move from one life stage to another was remarkably affected. For instance, BSF feed with high protein content diets, performed poorer than the control group with a balanced diet. This was confirmed in a later study of Danieli et al. [49]: high protein diets were observed to have poorer yields. In addition, De Smet et al. [48] stated that high fibre diet lead to a cease on insects' development. This was supported by a paper published in 2021 by Norgren et al. [50]: substrates rich in complex carbohydrates (cellulose or mainly lignocellulose) are not appropriate for BSF diets. Finally, in the study of Liu et al. [51] the impact of lignin on BSF larvae, present in the diet, was determined. They concluded that all types of fibre addressed in their study (ADF, NDF, hemicellulose, cellulose, lignin) had a negative effect on larval growth (except hemicellulose), with lignin having the strongest impact.

In a Master thesis written by Michael Woods [52], it is stated that most of insects required 9 or 10 amino acids to be included in the diets (lysine, tryptophan, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine, and arginine). However, although these are essential; and it is crucial to include a balanced mix of amino acids on their diets, it seems that a feed including this mixture of amino acids has not been specifically designed as per yet. The same authors state that the role of lipids has been underestimated in insect nutrition, which seems to be undersupplied in most of the diets. Particularly of relevance are sterols, which need to be from the right source (i.e. plant or animal) to satisfy insect requirements, and are essential nutrients since insects are not able to produce them. Similarly, the knowledge about vitamin requirements is very scarce [51], but it is usual that grains or seeds employed for diets do not contain enough vitamins, or none at all. It is then recommended to use fresh fruits or green tissues to supply water soluble vitamins, mainly ascorbic acid, when essential for the insects. Regarding lipid soluble vitamins, insects only need to get Vit A and Vit E from the diet. Finally, mineral nutrition is the most unknown for insect diets. They need sodium, potassium, phosphate and chloride; but don't have equal requirements to vertebrates regarding iron or calcium.
Experimental work in the Master thesis of Woods demonstrated that inclusion of sterol (from pork brains) yielded lower number of larvae, but significantly larger; also, that inclusion of blood meal (10% w/w) increased survival of the larvae [52]. Although manures are often considered the main feed there is a good potential to use green wastes (plant material) or non-food plant sources, e.g. seaweed [53], although in that work inclusion rates below 50% were optimal while still increasing omega -3 content of the BSFL.

It is clear that little work has been done on the nutritional requirements for insects to grow and develop in optimal conditions. Only a balanced diet incorporating all the essential nutrients will provide optimum results. In spite of a comprehensive search conducted over several data basis, we could not find enough papers related to dietary requirements for BSF. The data bases employed were Google Scholar and Science Direct. The keywords are summarized in following table (Table 1). Publication date was not limited to expand the search. The most relevant papers were related to the protein carbohydrate ratio. No paper was found regarding the essential nutrients that BSF may require in order to proper grow and develop. It needs to be noted than only the first 50 results coming from the data base search were reviewed.

Table 1: keywords of literature search on BSF nutritional requirements

Data base	Keywords	Papers found	Papers with the right information
Google Scholar	"black soldier fly" AND dietary AND requirements	8930	1
	"black soldier fly" AND limiting AND nutrients	3300	1
	"black soldier fly" AND requirements AND nutrients	7640	1
Science direct	"black soldier fly" AND dietary AND requirements	255	0
	"black soldier fly" AND limiting AND nutrients	156	0
	"black soldier fly" AND requirements AND nutrients	361	0

Other requirements regarding diet

Interaction with microbiota

The microbiota associated with the feed substrate and the insect gut seems to play an important role for substrate processing by BSF larvae. De Smet et al. [48], pointed out that many industries after sterilising the substrates found low conversion rates and growth. To overcome this they are inoculating the "clean" substrate with a small amount of "dirty" substrate from a previous rearing cycle. Larva gut microbiota are essential for efficient food conversion; it seems that a versatile core bacteria population supports the digestion of the substrate components. Supporting these results, Jiang et al. [54] found that the substrate may be colonised with bacteria coming from the insect gut, facilitating the digestion and reduction of persistent materials such as lignin or cellulose. As well as providing a diverse source of enzymes to digest the substrate the high pH of the gut appears to aid in sterilising the substrate [55]. This indicates that the gut microbiota are a specialised group that can persist in an extreme environment.

Investigations into the BSF gut microbiota has gained increased attention in the last years. As highlighted by Klammsteiner et al. [56], gut microbiota seems to be very stable over the larval phase, regardless the substrate employed. It needs to be mentioned, that substrates employed in this research had a very low bioburden load. It was suggested that the ability of larvae to generate antimicrobial peptides will act as a barrier for extrinsic bacteria colonisation, supporting the original microbial population diversity over time. Similar results were observed by Shelomi et al. [57], where two different substrates were fed to BSF (soy pulp and cafeteria waste); it was suggested that gut larva microbiome is highly preserved and the microbes were similar in a high percentage; in spite of the fact that the initial microbiome on the substrates were totally different. It has also been suggested that antimicrobial peptides are produced within the BSF gut, also as a tool to control the microbiome of the substrates. This is supported by work of Vogel et al. [58] who demonstrated diet-dependent changes in expression of antimicrobial proteins by BSF gut bacteria. They identified 53 genes for antimicrobial proteins. Most differential expression with the standard diet was due to a diet high in protein or oil, least change was observed with cellulose based diets. Also, these authors suggested that BSF maintains its own core microbiome, which is highly dependent on the location and feed, although it seems that remains somehow constant even if the diet is modified. Wynants et al. [59] described that although gut microbiome can adapt to the different diets, the similarities between substrate and gut population is very low.

Some authors support the theory that BSF gut microbiota can be manipulated through the substrate composition. Klammsteiner et al. [60], published that different diets can have an impact on gut microbiotal populations (when comparing olive and canteen wastes), however it was found that differences in the main metabolic pathways were not that clear. Authors indicated that larvae can use a specific “genetic toolkit” rather than expressing specific genes. It means, that although some changes are observed in microbiota populations, metabolic pathways remain stable.

Using companion bacteria to inoculate manure has been already discussed in the corresponding section, where it was demonstrated that these practises can improve the insect performance. However, it is not clear what the mechanism underlying this improvement is. As demonstrated by Kooienga et al. [61], bacterial supplementation needs to be evaluated at different production levels, since not all bacteria perform equally when compared to lab or pilot plant scale; this will lead to the use of targeted microbes for their industrial application.

In conclusion, it can be said that the gut microbiome may be affected by the diet, to some extent; however, gut population has no similarity with the microbe communities found in the substrate. There is a core microbiome, which remains stable and is dependent on insect location. It seems that, although some gut microbiome changes can be observed over time, the main metabolic pathways expressed by the gut microbiome remains constant. This is due to the relevant role that these microorganism play to provide nutrients to BSF larvae.

The potential for use of BSF to produce high value product was shown by Zheng et al. 2012 who used rice straw as a substrate for BSF growth but supplemented with a microbial inoculum to produce lava oil. While they called this biodiesel other researchers have used this as a nutrient for chicken rearing [43], in which it compared well with corn and coconut oils.,

Further research should be focused on identifying what microbiota species are present in the gut of very efficient insects in terms of growth and conversion ratios; as well as, what type of metabolic pathways are being provided for such microorganisms. Identifying them and how they help BSF to process substrate could be of help to design strategies to treat the substrates, which allow BSF to be more efficient when converting food wastes into high quality protein.

BSF feeding strategy

A recently published paper, focused not only of the substrate composition, but also on the feeding strategy to optimize feed conversion of black soldier fly larvae. In general, it was observed that providing continuous feed resulted in better conversion rate regardless of the substrate employed [62]. These results are supported by other research, in which it was proved that a batch feeding system showed the highest body weight [41]. In addition, a preliminary experiment of Barragan-Fonseca et al. showed that black soldier fly larvae fed all at once rather than three times per week reached a higher biomass [47].

It should be noted that nutritional content of BSF varies according to lifecycle stage. Liu et al. [63] found that maximum protein was achieved in the early larval stages, maximum fat was achieved at the pre-pupal stage.

Potential diets for black soldier fly rearing

Conventional diets

Even though it is not designed specifically for insects, commercial chicken feed is often used as a reference diet for black soldier fly, when reared for research purposes [17,19,21,64]. Furthermore, black soldier fly larvae are reported to perform well on diets developed for the rearing of *Musca domestica* such as the Gainesville diet (50 % wheat bran, 30 % alfalfa meal, 20 % corn meal) [65]. Therefore, this diet is also traditionally used as a standard [21,32,66,67]. It has been reported that both diets are equally suitable for rearing black soldier fly [68].

Diets based on low-valorised by-products

When referring to low-valorised products this means by-products or side streams from the food/feed industry that are composted, wasted or used for purposes such as anaerobic digestion, incineration and land-filling. Higher valorisations of these products might be possible, such as usage in insect feed. However, these type of diets often face challenges such as legal restrictions. For example, insects reared within the European Union are considered to be "farmed animals" (Reg. 2017/893), and, therefore, several restrictions are applied for their diets (Reg. (EC) 1069/2009). Hence, it is prohibited to use certain materials such as slaughterhouse or rendering-derived products, manure, catering waste or unsold products from food industries or retailers that contain meat or fish. However, legal restrictions will not be taken into account in this section as this is not the scope of this literature review.

Black soldier fly larvae have been indicated as one of the most appropriate insect species for the valorisation of low-value organic by-products [69,70]. For several reasons as discussed above, i.e. sustainability, economics, diets containing low-valorised ingredients are considered the most promising for BSF mass rearing. In a review paper published in 2018 [48], the huge variety of diets that have been tested with BSF (from fruits waste, to manure or seaweeds or animal cadavers) were highlighted as well as how the insects were able to grow in all of them.
As BSF rearing on by-products has been widely explored, this review will mainly focus on streams with highest potential to be used as BSF feed.

Catering waste

One of the most promising side stream based diets for black soldier fly larvae rearing is 'swill', i.e. catering waste. In a recent study of Veldkamp et al. [64] different biowaste diets were tested, i.e. solid pig manure, swill, olive pulp, pig manure liquid mixed with chicken feed and silage grass. Chicken feed was used as a reference diet. The study reports significantly higher growth rates of larvae reared on swill (13.4 mg/day) than found for the reference diet chicken feed (7.2 mg/day). Furthermore, the waste reduction index of larvae reared on swill as well as the ECI (efficiency of conversion of ingested food) was higher than found on any of the tested diets including the control (Table 2). The larvae fed with swill were able to convert the substrate completely into larval biomass and frass.

Table 2: Overview of relevant results (catering waste) from the study of Veldkamp et al. (2021) [64]

Substrate	Day of harvest	ECI (g:g DM)	Waste reduction index (DM g/d)	Larval growth rate (mg/d)	Larval end weight (mg)
Chicken feed (control)	8	0.34	4.5	7.2	69
Swill	8	0.46	10.6	13.4	119

Manure

An overview of the different studies discussed in this section is presented in Table 3**.**

Manure has been broadly studied for the last 25 years as a promising substrate for the rearing of BSF; although earlier research can be found in the 1970s. Such research was based on the fact that BSF uses manure as a substrate in nature, and therefore, seemed to be a good avenue to reduce the impact of farming on the environment by recycling manure into a more nutritive biomass.

In the research of Miranda et al. [71] manure from swine, dairy and poultry were compared as BSF substrates with Gainesville diet (50 % wheat bran, 30 % alfalfa meal, and 20 % corn meal). Results showed differences in parameters for larvae fed different manure types. The authors stated this is probably due to the difference in nutritional value of the manure types. In general, larvae fed dairy manure performed poorer in terms of development time and survivorship compared to those fed poultry or swine manure. Larvae fed the control Gainesville diet had the shortest development time, the highest survival rates, and produced the heaviest prepupae. However, as shown by the growth curve presented in the study, larval end weight did not differ significantly across larval diets (Figure 1).

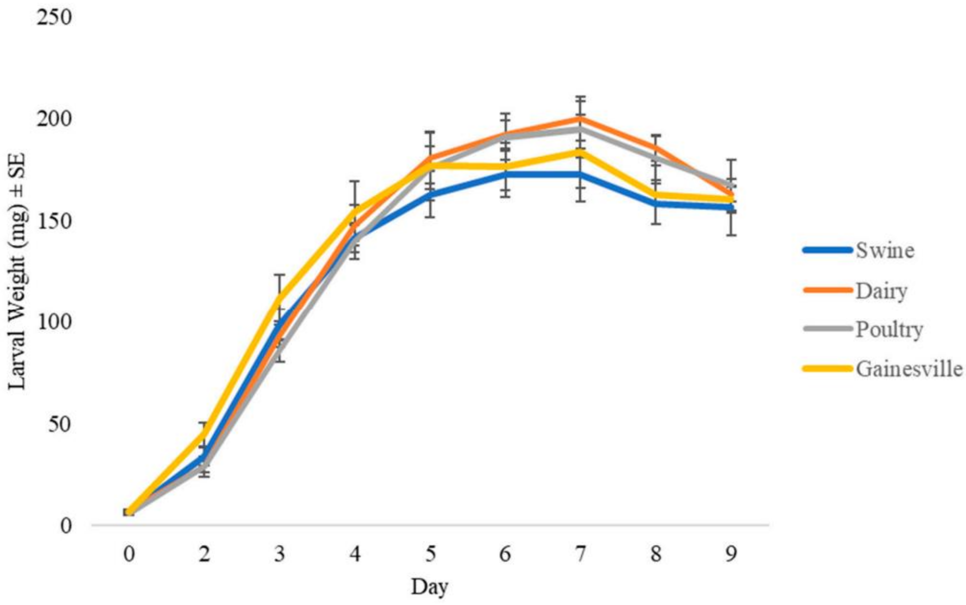

Figure 1: growth curve of larvae produced on manure and control diet, derived from de study of Miranda et al. [71]

In the study of Broeckx et al. [19] 12 different side streams were tested as potential substrate for the rearing of BSF, including chicken manure. Treatments were compared to two control diets: chicken feed and Gainesville diet (Table 4). Larvae fed chicken manure performed better than the control Gainesville diet in terms of bioconversion efficiency (significant 15.43 % ± 1.65 %VS 7,72 % ± 1.46 %), FCR (2.00 ± 0.33 VS 3.43 ± 0.74) and larval mean maximum weight (significant, 134.9 mg ± 11.8 VS 83.8 mg ± 13.8). Larvae reared on the control chicken feed performed better than larvae fed chicken manure in terms of bioconversion efficiency, FCR and larval mean maximum weight , but no significant differences were found.

Regardless of the potential, there are still some unknowns on how manure properties (physical and chemical) are affecting BSF performance and efficacy and further optimisation of this potential diet is needed.

Some authors reported that mixing manures of different origin may be of benefit. For instance, since dairy manure is too high in fibre and low in nutrients, it can be mixed with chicken manure, and it was suggested that a ratio of 40% beef/ 60% chicken manures was optimal for conversion and manure mass reduction. However, no detailed information about initial manure compositions was provided, it was just reported that C/N proportion was higher for dairy manure compared to chicken (21.8 vs 15.7), which may be indicative of higher fibre content [72]. This research provided the information required to confirm that BSF is able to degrade cellulose, hemicellulose and lignin, with better efficiency when the C/N ratio is kept around 14 with an initial pH of 7.8. Further research from the same authors confirmed that using assisting bacteria (showing exogenous lignocellulosic activity) could further improve the outcomes for survival, development time and efficiency [73]. In this line of research, it has been reported that bird manure may be a better substrate for BSF in terms of feed conversion, but also on the quality of the amino acid profile of the insect when used as feedstuff [74]. When quail manure was added to regular wheat bran to feed BSF, it was observed that a 40% inclusion of manure led to a significant improvement on insect performance, but also to a different body mass composition (higher amounts of w-7 and w-p fatty acids were detected) [74].

Trying to optimize the use of manure, and to overcome some potential lack of nutrients, other food by-products have been used to reinforce manure composition, to avail for better BSF performance. In a study conducted in 2007, cow manure was mixed at different ratios with fish offal [75]. The larvae collected at the end of the study had a significant higher body weight when a 25% of fish offal was incorporated. However, once again, a detailed description of the substrate composition was not provided [75]. Horse and sheep manure were supplemented with vegetables waste (ratio 1:1) and compared to 100% manure. In general, body weight was increased when the mixture was employed, with better results for sheep manure with the incorporation of vegetable wastes. This substrate was determined to be the highest in protein content and the lowest in fibre content. Mortality was not remarkably affected, but for 100% sheep manure, where it was higher than the other substrates [76]. Rehman et al. [77], incorporated soybean curd residue to dairy manure. The authors suggested that dairy manure, due to its high fibre content, might be difficult to digest by BSF. Results concluded that an optimal C/N ratio needs to be found for a proper BSF larvae growth, along with initial pH (as reported by other authors). In this case, a 16.2 ratio and initial pH of 6.7 was found to be the best one among the different treatments tested. However, this optimal ratio may vary depending on the type of manure and the type of supplement provided. It was highlighted that high fibre content also contributes to the pH buffer capacity of the media, to keep this parameter stable over the rearing process, which may be of benefit [77]. Based on the data provided, our interpretation is, that BSF may have a more balanced intake of protein and fibre as energy sources at lower C/N ratio, i.e. if organic nitrogen is available, this may lead to a better feed conversion and bioconversion efficiency. In this regard, Cammack et al. [78] studied the impact of the moisture level, protein content and carbohydrates content in BSF development. Moisture was determined as the main factor, with a value of 70% being preferable. On the other hand, a balanced diet (21% protein and 21% carbohydrate) lead to the fastest larvae development: however, all experimental diets performed poorer than control (Gainesville diet), indicating that other factors are playing a role, such as associated microbiome, since these diets were artificially manufactured.

A more recent approach, is to inoculate the manure with companion bacteria. Mazza et al. [79], used strains isolated from BSF eggs and gut. Media Inoculated with a combination of four bacteria was found to promote higher weight gain, highest manure reduction and increased crude protein content in the final BSF biomass. However, why bacteria are improving the BSF performance is unclear.

In a very recent review paper [80], it was highlighted that BSF efficiency to convert manure into insect biomass depends mainly on manure temperature and pH, type of manure, if a co-digestion process is taking place and the strain of *H. illucens* used, as well as the population densities employed. Optimal temperatures were found to be in the range of 27 °C to 30 °C; at the lower temperature range females were heavier, but it took 4 days more for them to complete larval and pupal development [81]. Regarding pH, an initial value of around 8 was stated as optimal in terms of larva development ratio, weight gain and development time. Although pH may change over time, due to the insects activity [40].

Table 3: Overview of relevant results (manure) from different studies discussed

Reference	Substrate	Developing time (days)	Bioconversion (%)	Feed conversion rate	Substrate reduction (%)	Mortality (%)	Dry mass(%) of live weight BSF
Ur Rehman et al. (2017) [77] **¥**	Dairy Manure and chicken manure (mixed at different proportions)	18-22	4.2-9.9	5.6-10.3	43-55	1.6-10.5	19.5-23.2
	Dairy manure and soybean curd	19-23	6.3-15.2	3.2-6.2	26-72	1-9	21.4-26.5
Myers et al. (2014) [82] **¥**	Dairy manure	26-30	NA	NA	35-59	15-29	NA
Alyokhin et al. (2018) [83] **¥**	Horse manure	25.75	NA	NA	NA	NA	NA

Miranda et al. (2020) [71]	Poultry manure	14	NA	NA	46	22 (% of prepupation)	NA
	Dairy manure	16	NA	NA	48	55 (% of prepupation)	NA
	Swine manure	15	NA	NA	33	27 (% of prepupation)	NA
	Gainesville	13	NA	NA	NA	12 (% of prepupation)	NA
Ur Rehman et al. (2019) [73]¥	40 % dairy and 60 % dairy manure with bacteria	19	10.8	4.5	48.7	0.9	NA
El-Dakar et al. (2021) [74]¥	Quail manure	30	NA	NA	NA	NA	NA
	Goat manure	26	NA	NA	NA	NA	NA
	Poultry manure	26	NA	NA	NA	NA	NA

Julita et al. (2018) [76]¥	Horse manure	22.6	NA	NA	NA	26.4 (prepupal)	NA
	Sheep manure	26.0	NA	NA	NA	32.4 (prepupal)	NA
Mazza et al. (2020) [79]¥	Chicken manure	NA	7.42	NA	49.2	NA	NA
	Chicken manure + single bacteria	NA	7.3-8.8	NA	46.7-53.6	NA	NA
	Chicken manure + poly-bacteria	NA	8.0-10.4	NA	48.6-52.9	NA	NA

¥: No control diet was used. NA: not reported.

Table 4: Overview of relevant results (manure) from the study of Broeckx et al. (2021) [19]

Substrate	Time to reach max. weight (days)	Bioconversion Efficiency (%)	FCR	Waste reduction (%)	Survival (%)	Maximum mean larval weight (mg)

Gainesville (control)	8	7,72 ± 1.46	3.43 ± 0.74	45.9 ± 5.7	97.2 ± 1.8	83.8 ± 13.8
Chicken feed (control)	7	17.56 ± 3.37	2.08 ± 0.35	49.9 ± 9.6	96.3 ± 1.9	148.4 ± 21.8
Chicken manure	8	15.43 ± 1.65	2.00 ± 0.33	36.4 ± 13.1	97.7 ± 1.4	134.9 ± 11.8

Brewery by-products

In the study of Jucker et al. [84], different diets based on brewery by-products were tested and compared to the standard diet (mixture of 50 % wheat germ, 30 % alfafa, 20 % corn flour + water): 1) brewers' spent grain, 2) chopped brewers' spent grain, 3) trub[1] and 4) mixture of brewers' spent grain and trub (1:1). The larval growth curve and end weight presented in the study shows no significant difference between the control diet and the mixture of brewers' spent grain with trub. Also larval development time of the mixture diet was significantly similar to the standard diet, with a mean of 18.7±0.3 days to reach prepupal stage. Other tested diets were found less suitable for BSF rearing. Nevertheless, authors stated that BSF was able to feed, survive and complete its lifecycle on all tested diets.

Table 5: Overview of relevant results (brewery by-products) from the study of Jucker et al. (2019) [84]

Substrate	**Larval development (days)**	**Mortality**	**Final larval weight (mg)**
mixture of 50 % wheat germ, 30 % alfafa, 20 % corn flour (control)	15.3 ± 0.3	low	235 ± 0.002
Brewers' spent grain	24.7 ± 0.3	Comparable to control	86 ± 0.003
Chopped brewers' spent grain	33.0 ± 2.3	Comparable to control	87.3 ±0.007
trub	28.3 ± 1.9	Highest mortality (significant)	180 ± 0.008
brewers' spent grain + trub (1:1)	18.7 ± 0.3	Comparable to control	244 ± 0.011

In the study of Broeckx et al. [19], beer draff was tested as potential diet for BSF rearing and compared with two standard diets: chicken feed and Gainesville diet. Maximal mean larval weight of larvae fed beer draff did not differ significantly to larvae fed the control chicken feed. Maximal mean larval weight of larvae reared on beer draff was found significantly higher (130.9 mg ± 19.6) than larvae fed the control Gainesville diet (83.8 mg ± 13.8). In terms of bioconversion efficiency, larvae fed beer draff did not differ significantly from larvae fed chicken feed, however, larvae fed beer draff showed significantly higher bioconversion efficiency (13.91 ± 0.40) than larvae reared on the Gainesville diet (7,72 ± 1.46). The authors reported a lower development time (the number of days between the start of the experiment and the day of harvesting) for larvae fed beer draff (7 days) than larvae fed the Gainesville diet (8 days).

[1] trub is the term used for the material, along with hop debris, left in the whirlpool or hopback after the wort has been boiled then transferred and cooled

Table 6: Overview of relevant results (brewery by-products) from the study of Broeckx et al. (2021) [19]

Substrate	Time to reach max. weight (days)	Bioconversion Efficiency (%)	FCR	Waste reduction (%)	Survival (%)	Maximum mean larval weight (mg)
Gainesville (control)	8	7,72 ± 1.46	3.43 ± 0.74	45.9 ± 5.7	97.2 ± 1.8	83.8 ± 13.8
Chicken feed (control)	7	17.56 ± 3.37	2.08 ± 0.35	49.9 ± 9.6	96.3 ± 1.9	148.4 ± 21.8
Beer draff	7	13.91 ± 0.40	2.32 ± 0.17	46.2 ± 10.4	95.2 ± 3.1 a	130.9 ± 19.6

Emissions

As insects are considered an alternative protein source, it is relevant to map the emissions related to insect production, especially when reared on side streams. It is well known that livestock agriculture (i.e. ruminant, pig and poultry) is a significant contributor to the production of greenhouse gas emissions. The FAO has reported that total emissions from global livestock equate to 7.1 gigatonnes of Co2-equiv per year [85].

In the literature search of work package 1 (activity 3) the emissions related to the rearing of *T. molitor*, *A. domesticus* and *L. migratoria* as well as measurement of emissions are discussed. Relevant emissions include carbon dioxide, nitrous oxide, ammonia and atmospheric particulate matter. Methane was found irrelevant for the species in scope as earlier studies showed no or minor methane production was detected for *T. molitor*, *A. domesticus* and *L. migratoria* [86–88]. While exploring the insect emission topic, it became clear that research and data is often lacking or even missing. For example, no data was found on emissions during a full rearing cycle.

Building on the information of the WP1Act.3 report, the literature search will be expanded with inclusion of the black soldier fly insect and emissions relevant for this species. The focus is on direct emissions, i.e. originating from metabolic processes. Black soldier fly larvae can be reared in an array of substrates that vary in moisture content, pH, C/N ratio, etc. Furthermore, they exhibit a complex interaction with microbiota that develop simultaneously. Not only will the starting conditions vary, but the substrate will evolve over time as well, exhibiting variable emission patterns. Measuring methods and techniques of these emissions are included.

Table 7: Overview of the literature regarding emissions related to BSF rearing

	Substrate	FCR (dry feed/kg live larvae)	CO_2 (g/kg dry matter larvae biomass)	CH_4 (mg/kg dry matter larvae biomass)	N_2O (mg/kg dry matter larvae biomass)	N (g/kg dry matter larvae biomass)	Rearing conditions	Duration
Mertenat *et al*. (2019) [89]	Kitchen waste (fruit and vegetable raw peeling as well as cooked food remain such as rice and vegetables. Carbon-to-nitrogen ratio (C/N) of 15–20			5.5	118		10,000 larvae per 15 kg substrate (in 3 feedings) in 60 x 40 cm crates	
Guo *et al*.(2021) [90]	Food waste from households, restaurants and canteens mixed with rice hull powder (ratio 4:1)					3.5	14,000 larvae per 25 kg substrate (daily feeding) in 30 x 26 cm crates	

Parodi *et al.* (2020) [91]	7% ProtiWanze®, 47% DB-Blend and 6% binding agent, and had an acid pH (near to pH 4)	2.2	2750 ± 314	28 ± 29	53 ± 27	1.2 ± 0.7	10,000 larvae per 4 kg substrate (fed once) in 50 x 30 crates	7 days
Pang *et al.* (2020) [92]	Food waste from restaurants mixed with chopped rice straw (ratio 9:1) and pH correction (3.0, 5.0, 7.0, 9.0 and 11.0) (35% DM)	17.6 3.9 3.4 2.9 2.4	4339 1745 1377 1058 603	9.8 8.4 11.3 21.3 9.2	81.1 5.6 8.0 7.6 1.4	6.1 2.6 8.2 11.2 8.7	1,800 larvae per 1.2 kg substrate (fed once)	10 days
Ermolaev *et al.* (2019) [93]	Local food waste (sourced from a canteen) (25 % DM)	1.4	1750 ± 170	49 ± 29	21 ± 13		700 larvae per 1.18 kg substrate (in 3 feedings) in 21 x 17 cm crates	14 days
Parodi *et al.* (2021) [94]	Pig manure which consisted mainly of fresh faeces (i.e., maximum 2-day old) that	2.2	1956 ± 105	10,066 ± 2,652	6 ± 14	58 ± 7	20,000 – 25,000 larvae per 18.83 kg substrate (fed once) in 100 x 50 cm crates	8.7 days

	were in contact with the urine present on the floor (23.9 % DM)							
Pang *et al*. (2020) [95]	Pig manure and corncob mixtures with a varying C/N ratio (15, 20, 25, 30, 35) (30% DM)	4.6 4.2 3.8 4.1 4.9	1409 1517 1636 1732 1726	9924 9202 5285 1746 1111	13.4 13.6 9.8 13.7 18.9	12.6 7.8 5.1 3.3 1.6	1,600 larvae per 1.0 kg substrate (fed once) in 3 L glass bottles	12 days
Chen et al. (2019) [96]	Fresh pig manure and corncob (2.2:1 ratio ww basis) with a varying moisture content (45%, 55%, 65%, 75%, 85%)	315.3 67.4 16.4 6.5 18.3	30190 12520 5609 3556 6160	937 385 257 744 199,989	81.1 17.3 11.7 1.7 13.1	548.3 136.2 25.0 9.6 21.0	450 larvae per 159.5 g dry matter in 3 L glass bottles	8 days

Carbon dioxide

Due to the active microbial population that grows alongside the black soldier fly larvae in the rearing substrate, observed carbon dioxide emissions are always a combination of the metabolic activity of both. Parodi et al. [91] estimate that microbial metabolism contributes for 34% of the total carbon dioxide emissions. The median of all published data reported in Table 7 amounts to 1745 g of CO_2 per kg of dried larvae, which is in the same order of magnitude of that of the results published for other insect species such as *Tenebrio molitor* (1031 g CO_2), *Acheta domesticus* (1468 g CO_2) and *Locusta migratoria* (734 g CO_2) [86].

Measurement

For the measurement of the carbon dioxide concentration in the air, different techniques are available and all techniques have both advantages and disadvantages.

- The NDIR CO2 sensor works with a NDIR (non-disruptive infrared) light source of 4.26 µm. On this basis with the amount of light (or the lack of light) that reaches the sensor the CO2 concentration can be determined. Cross-sensitivity with water vapour (humidity) is reduced by using this light frequency [97].
- Electrochemical sensors make use of a chemical reaction that is caused inside the sensor if carbon dioxide is present. The disadvantage is that the sensor can drift or lose accuracy after some time [98].
- A Metal-oxide carbon dioxide sensor is a sensor that uses the resistivity of metal to test the amount of carbon dioxide in the air. This is a very simple (and cheap) technique but it is prone to temperature, humidity, other gases in the air and drift. Usually it is used for higher concentrations and uses ABC (Automatic baseline correction) [99].

In scope of WP1 of the ValuSect project, an accumulation chamber has been made. This accumulation chamber initially contained an electro-chemical CO_2 sensor with a measuring range of 0-10%. When insect emissions were measured, emissions were detected only in the lower measuring range of the sensor (0-1%). The inaccuracy of sensors is dependent on this range: a broader measurement range equals a larger inaccuracy. This made the initial sensor unsuitable for measuring insect emissions. As a result of these findings an extra CO_2 sensor was added with a suitable measurement range usable for the planned experiments. The sensor applied was the KCD-HP-100A-P (0-1%). As black soldier larvae are a new species with a less known emission pattern, both sensors (in the low and high range) can be used for emission measurements.

Nitrous oxide

Nitrous oxide has a significant effect as a greenhouse gas and arises during nitrification and denitrification. Published data on emissions by BSF larvae cover a broad range (Table 7) with values from 1.4 mg per kg of produced dry larvae up to 118. The work of Parodi et al. [94] found that BSFL grown on pig manure had a negligible nitrous oxide emission and no effect of treating the manure with BSFL was found.

The concentration of nitrous oxide in the air can be measured with electrochemical sensors. In comparison with the CO_2 emissions, the concentration of nitrous oxide emitted by insects is generally lower. Due to the limited emissions of nitrous oxide of the BSFL electrochemical sensors are not suitable. Measurement with NDIR sensors is more accurate. Another option is gas spectroscopy but this brings more sophistication.

Before sensors can be selected a mass balance / simulation has to be made to determine the expected measurement ranges required. When the measurement ranges expected are above 10-20 ppm electrochemical sensors can be selected. If the expected measurement ranges are below 10-20 ppm gas analysis using gas spectroscopy are more suitable.

If the mass balance indicates low emissions of nitrous oxide, measurement is not possible with the use of electrochemical sensors and the measurement has to be executed with gas spectroscopy.

Ammonia

The microbial degradation of proteins catalysed by ureases leads to the formation of the soluble ammonium (NH_4^+) and the more volatile ammonia (NH_3). The equilibrium between these two components is largely influenced by pH (pKa = 9.25). In an acidic environment ammonium is the predominant form, in alkaline conditions this equilibrium will shift in favour of ammonia. Ammonia emissions that result from BSF rearing have been described [90–92,94–96]. Reported values range from 1.2 up to 548.3 g of N per kg of larvae produced (on a dry matter basis) with a median value of 8.45. However, the highest reported value is inflated due to poor larval growth (i.e. the high feed conversion ratio). In general, ammonia emissions tend to be highest in the second half of larval growth. It is hypothesised that this is related to larval activity. As growing black soldier fly larvae tend to steer the pH of their surroundings up to 9 [41,92], more and more of the ammonium is volatilized as ammonia. Pang et al. [95] was able to reduce ammonia emissions from larvae reared on pig manure by increasing the C/N ratio by mixing the manure with corncob. When nitrogen is not present in excess, larvae will be able to incorporate it in larval protein. Nitrogen compounds that are not incorporated in the larvae are exposed to microbial degradation and could eventually volatilise as ammonia.

From a literature search performed within WP1 of the ValuSect project, the ammonia concentration related to the rearing of mealworms and crickets was expected to be low: too low to be measured with electrochemical sensors. Only measurement using gas spectroscopy was possible, however, this could not be performed within the project. Nevertheless, it must be noted that black soldier fly larvae might emit higher concentrations of ammonia due to differences in substrate properties (compared to mealworms and crickets) as well as a broader range of low-valorised substrate possibilities (i.e. manure, wastes). A mass balance has to be drawn up in order to determine which technology is suitable for measuring emissions related to the rearing of this insect species (i.e. electrochemical, NDIR or gas spectroscopy).

Methane

Methanogenesis is a process that takes place under anaerobic conditions and is catalysed by specific microorganisms from the domain Archaea. Most methanogens are active in a more neutral pH range and methanogenesis decreases drastically below a pH of 6.2 [100]. Anaerobic conditions could occur in the liquid feed that is given to the black soldier fly larvae. When the required microorganisms are present, methanogenesis could therefore happen. On non-manure substrates, methane emissions ranged from 5.5 to 49 mg CH_4 per kg dry larval biomass [89,91–93]. However, on substrates that contained manure, values ranged from 257 up to 199,989 mg CH_4 per kg of dry larval biomass [94–96]. These results, and especially the high value, should be interpreted with care. The moisture content of this treatment was increased up to 85%, which resulted in poor larval growth and high methane emissions, inflating the reported methane emitted on a larval basis. Usually methane emissions are highest the first few days after inoculating the substrate with larvae, to gradually decrease afterwards. It is hypothesised that in the first few days larval activity is still rather low, which increases the risk for anaerobic conditions. When the larvae grow larger, the aeration of the substrate will improve and conditions for methanogenesis deteriorate. When starting conditions are too acidic (which is often the case for agri-food by-products), methanogenesis should be low.

The presence of methanogenic microorganisms is especially likely for substrates that originate from the guts of other farmed animals. Despite the considerably higher observed methane emissions, manure should not necessarily be excluded from the list of suitable substrates for rearing black soldier fly larvae. Treating manure with black soldier fly larvae is sometimes even proposed as a way of decreasing methane emissions that would otherwise naturally occur from biodegrading manure. Matos et al. report a decrease of 51.7% of methane emitted by cattle manure and 85.8% for pig manure [101]. However, Parodi et al. observed no significant differences between treated and non-treated pig manure [94].

The measurement of methane was out of scope for WP1 of the ValuSect project, since mealworms and crickets were not reported to emit these in high quantities (referring to the literature report). However, as mentioned above, it must be noted that the black soldier fly insect might emit methane due to different substrate types and properties (e.g. moisture content) compared to crickets and mealworms. Before a suitable sensor for measuring the methane concentration in the air can be selected a mass-balance needs to be executed to determine the needed sensor measurement range. When the range is known the correct sensor technology can be selected.

Odour

It must be considered that rearing black soldier fly larvae on side stream based diets might results in odour problems, which should be managed properly. This needs special consideration for organic wastes rich in nutrient and water content, as these are prone to faster dissolution. The emission of odours around an insect rearing farm can be inconvenient for their direct neighbours. For example, ammonia has been reported to cause odours and even to create a hazardous environment for workers in fly larvae composting and can represent a major pathway for nitrogen losses from the compost produced [20,102]. Nevertheless, Beskin et al. [103] found that larval digestion of organic substances can greatly reduce an array of volatile organic compounds (VOC's) that are known to be odorous. A reduction of 87% of these VOC's was observed in digested versus undigested manure. However, this study did not quantify VOC's that could arise during larval digestion.

In Europe the standard for odour measurements is stated in the EN-13725 [104]. This standard was first published in 2003 and revised in 2022. This standard describes the measurement of the determination of the odour concentration of an odorous gas sample. The unit of measurement is the European odour unit per cubic metre: ouE/m3. This is measured by determining the dilution factor required to reach the detection threshold. The due to the pre-dilation measurement range starts at > 10 ouE/m3 inluding pre-dilation and only usable at the source. When the odour concentrations are <10 OuE/m3 the odour concentration can be determined with the use of field inspection. This method is defined in the EN16841-1:2016 [105].

In the field measurement method a grid is created around an odorant source. The odour hour frequency for a grid square is determined. Each measurement point is measured repeatedly (for example 26 times at regular intervals over a one-year period). One single measurement result in the test is labelled as 'odour hour' or 'non-odour-hour'. Eventually the odour concentration can be calculated [106].

Dynamic Olfactometry is the standardized method of measuring odour by the European Standard EN. Due to the standardisation this method can be used to compare the odour of BSF breeding to other livestock, landfills, wastewater treatment plant or other odour emissions processes. However, this method includes different samplings during various periods and consequently is a more expensive method. A possible alternative is using an Electronic noses (E-nose), however, this method is not yet standardized [107]. An e-nose uses an array of electrochemical sensors to determine the individual concentrations of gasses present in the (sample air). These concentrations of gasses are not directly usable for an indication of the perceived smell by a human nose [108]. However when electrochemical sensors are used the measurement ranges and accuracy's of those sensors have to be taken into consideration.

Automation of the BSF production process

As industrial black soldier fly production is a relatively new practice in the EU, automated machineries and technologies are currently lacking or in stage of development. This development is a long process as this requires research and understanding of insect needs and behaviour. For instance, process monitoring to determine efficiency losses along the whole production line is crucial for process optimization. This includes determining the overall insect yield (i.e. viable eggs, survival rates, yield/crate). Furthermore, managing insect densities and feeding practices are also important process elements to be optimal [24].

Some insect producing and engineering companies today are already developing automated insects systems, however, these designs are mostly kept secret. Only a couple of engineering companies we know of are interested in building an industry scaled system [109–112]. They offer fully automated breeding systems but as expected, they are not keen to share information about the exact process and machines that they use. Most of these systems are patented or patent pending.

Table 8: keyword search on BSF automation

Data base	Keywords	Papers found	Papers with the right information
Google Scholar	"black soldier fly" AND "fully automated ""	70	2
	"black soldier fly" AND "autonomous breeding"	0	0
	"black soldier fly" AND "autonomous system"	0	0
	"black soldier fly" AND "automatic breeding system"	0	0
	"black soldier fly" AND "automatisation"	4	0
	"black soldier fly" AND "automatic rearing"	2	1? (In Korean)
	"black soldier fly" AND "automatic breeding"	1	0
Sciencedirect	"black soldier fly" AND "fully automated ""	6	0

	"black soldier fly" AND "autonomous breeding"	0	0
	"black soldier fly" AND "autonomous system"	4	0
	"black soldier fly" AND "automatic breeding system"	0	0
	"black soldier fly" AND "automatisation"	0	0
	"black soldier fly" AND "automatic rearing"	0	0
	"black soldier fly" AND "automatic breeding"	0	0

It is clear that currently information regarding insect automation is scarce (Table 8). However, it is very important that this is explored for the inclusion of insects in the society. Due to the lack of publicly available information on automated insect systems, production of insects currently requires much manual and labour-intensive interventions, increasing production costs of insect production and decreasing economic viability of insect production.

To demonstrate this, a BSF process flow is shown in Figure 2. Note that this process flow is an estimation based on the experience of the Inagro insects research centre. However, visualising the BSF process flow, makes it possible to identify the steps which have the most need of automation and thus currently require most manual labour:

Producing black soldier fly larvae is a process that requires a lot of inputs. These inputs are energy, water, feed and manual labour. Especially the latter is a bottleneck for making black soldier fly larvae (and its derived products) a competitive alternative protein source for (pet)feed manufacturers, especially in Western Europe where labour costs are increasing. In order to quantify the amount of labour that is needed in black soldier fly larvae production, the different steps that are required in this process were identified and required labour was estimated.

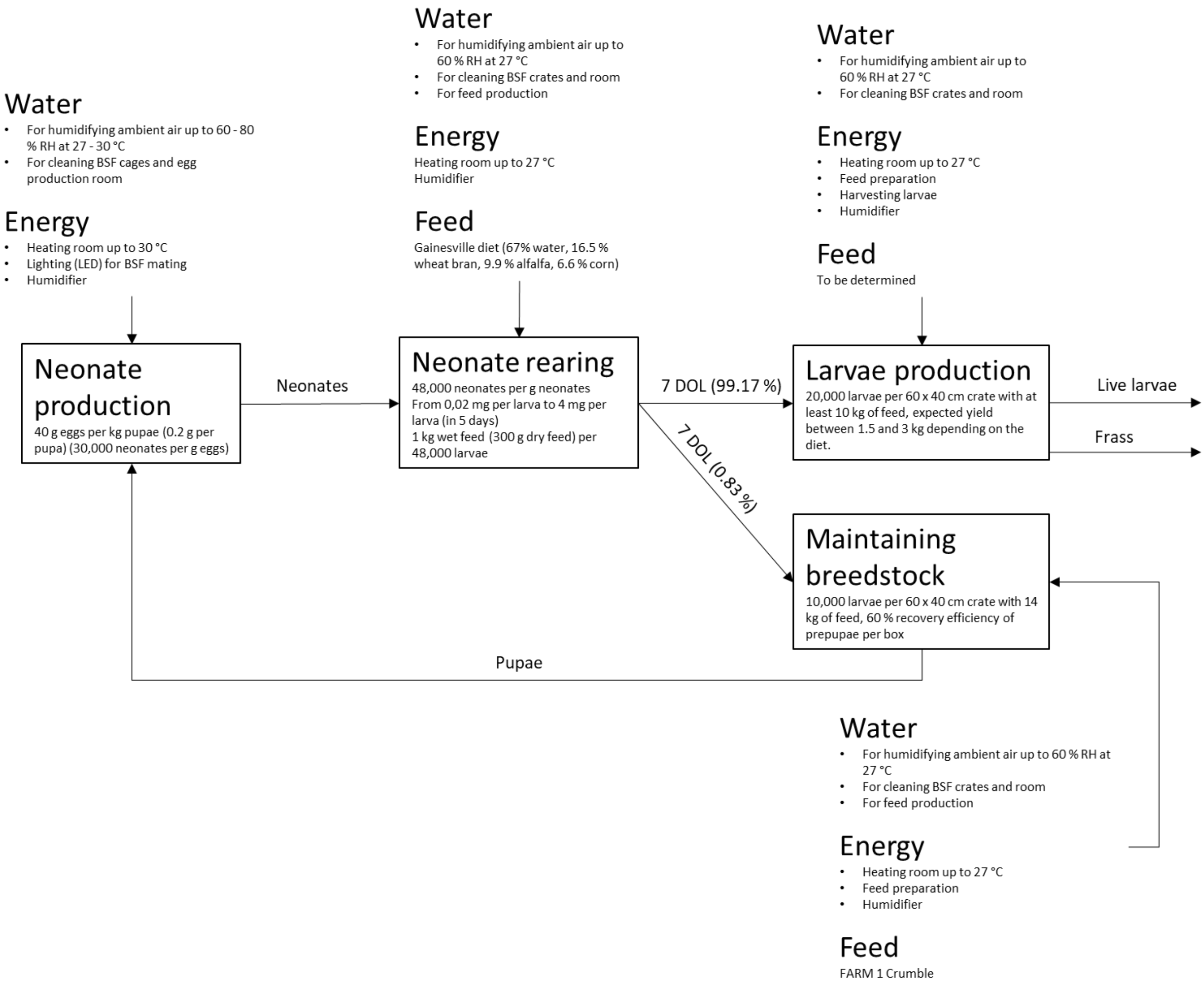

Figure 2: Process flow on how to produce black soldier fly larvae.

The time spent for each step in the production process is shown in Figure 3. The functional unit (U: 20,000 larvae of harvestable size) was chosen as such, to exclude the influence of diet induced differences in larval end weight. 20,000 larvae is approximately the amount of larvae for one rearing crate of 60 by 40 cm and is likely to yield somewhere between 1.8 and 3.0 kg of live larvae. It is estimated that in a predominantly manual production setting with little mechanical support around 16.5 minutes are needed per U. Larvae production accounts for over 65% of total labour. The influence of the other production steps is relatively less due to the fact that more FU's are produced in these steps. Most gains per U can therefore be made in the former. Within the larvae production step, the majority of time is needed to create batches of 20,000 larvae and to prepare a crate with substrate and add the larvae. Second is cleaning the crates after larvae production. The third most time consuming action occurs during neonate rearing, when the number of 5 day old larvae in a batch need to be determined. The authors recommend doing so, as it increases the control and lessens the variation later on during larvae production.

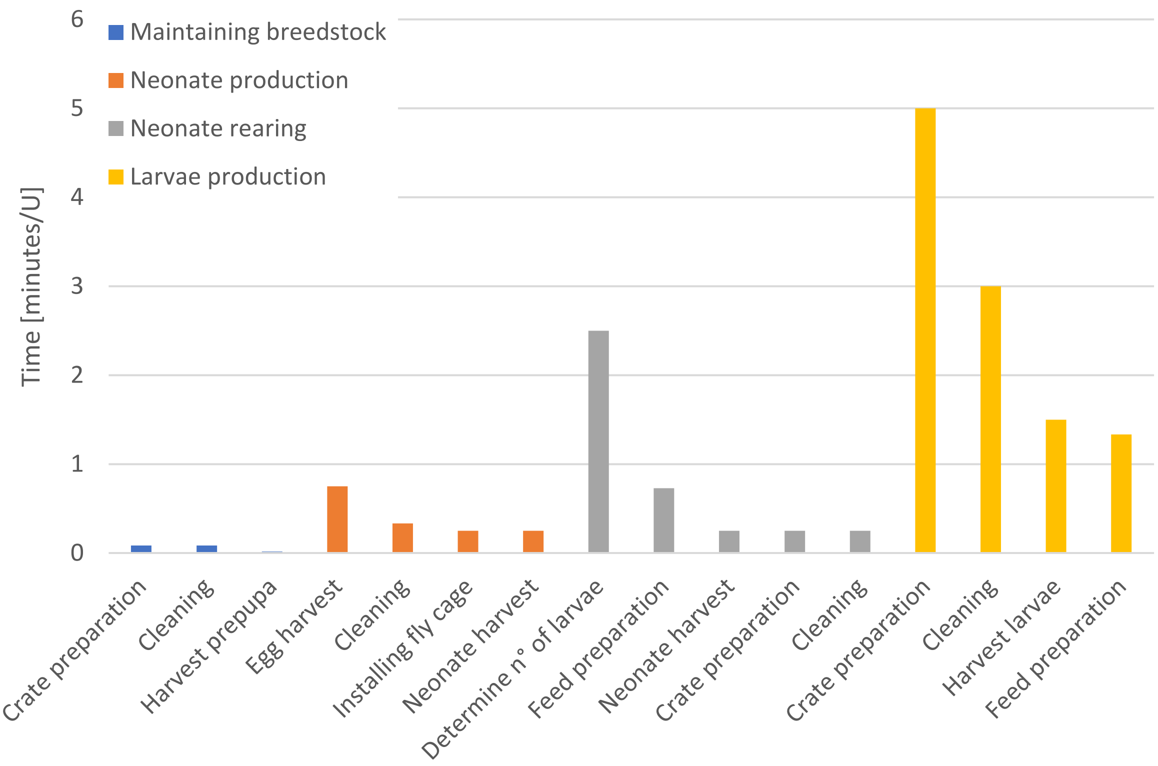

Figure 3: Time spend to produce 1 FU (20,000 larvae of harvestable size) for different aspects of a manual production system with minimal mechanisation.

Five steps that can be taken in order to reduce labour in the production process are:

1. Automated feed preparation and crate filling
2. Investing in a crate cleaning line
3. Larvae counter
4. Crate de-stacker and mechanical sieve, preferably with frass removal and larvae collector
5. Automated egg harvesting

Furthermore, technology to monitor and control the entire production process is needed.

Discussion

As insects have large potential as an alternative, sustainable protein source, they are expected to play an important role in future society. However, as insects for application in food and feed are a relatively new concept in the European Union, this sector in still facing some challenges. For instance, knowledge of insect behaviour, needs and production is still lacking, resulting in a current inefficient and non-optimal production. Consequently, insect-based (feed) products have not yet found their place in the market: costs are high (i.e. manual labour) resulting in an expensive product that is not yet able to compete with current feed protein sources, demand is low and rearing facilities are low-scale. Knowledge gaps for industrial insect production need to be filled in order to become optimised, sustainable and economically viable. Focus must be laid on lowering insect production costs in order to become lower end product prices, which can be done by the use of non-valorised side streams as insect feed, scaling up the processes and limiting manual operations [23–26].

In WP5 of the ValuSect project, we aim to start filling some of these gaps. We have performed this literature search in order to identify some knowledge gaps on insects for feed production, more specifically the black soldier fly. The identified knowledge gaps were categorised in 3 major topics:

- Black soldier fly diets
- Black soldier fly emissions
- Black soldier fly production automation

Black soldier fly diets

Identified as one of the major challenges for the insect production industry, is the development of optimal insect diets [23,25,113]. In order to get a better understanding of this topic, knowledge on black soldier fly dietary requirements was included in this literature review. We found that not much research was available on the **nutritional requirements** of black soldier fly larvae: the keyword search resulted in only a few papers on this topic. Furthermore, a large part of the available information addressed insects in general, and not specifically the black soldier fly. More information was found on the BSF diets abiotic parameters. In addition, some information could been found on the interaction of black soldier fly larvae with microbiota; it is clear this plays an important role in biomass processing by BSF, however, the interactions are currently marginally understood.

It is well known that BSFL can efficiently convert low-value substrates into high value biomass. Furthermore, life cycle analyses (LCA) indicate that the production of black soldier fly larvae can be done more efficiently and cost-effectively if the larvae are reared on low-value biomass [6,18,23,26,30]. Consequently, interest in the production of black soldier fly larvae on side streams has resulted in many studies being generated.
However, due to factors such as legislation, knowledge gaps & instability or storage issues of (fresh) biomass, in practice insects are often produced on diets not specifically designed for these animals. For instance, the use of chicken meal for BSFL. These diets do contain the required

nutrients, but often not in optimal proportions. This may lead to inefficient production and high production costs. In this literature review, two conventional diets were identified that are used as a BSF standard diet worldwide, i.e. Gainesville diet and chicken feed [65,68].

As the topic of BSF production on side streams has already been widely explored, the focus of this literature search was delineated to low-value diets with the most potential for black soldier fly mass rearing, i.e. biomass performing equally or better than the standard diets. Parameters such as FCR, bioconversion efficiency, larval end weight, insect survival & growth curves were taken into account. The literature search was not limited by legislation.

From this literature review, following low-value substrates came forward as potential feed for BSFL production:

- Catering waste
- Chicken/poultry manure
- Beer draff

Catering waste was found the most promising for BSF rearing of all listed [64]. Poultry manure and beer draff seemed to overall perform equally to chicken feed and better than Gainesville diet [19,71].

During the performance of the literature review on BSF production on side streams, another challenge came forward. Even though many publications on this topic were available, it was very difficult to compare results from different research groups. For instance, it is known that the insect rearing conditions highly impact research results, but often these rearing conditions are not or only partly mentioned in conducted studies. Furthermore, for insect feed experiments, diverse methodologies have been applied to quantify the efficiency of conversion (e.g. feed conversion ratio, bioconversion efficiency, etc.). Since there are now numerous methods described and consequently used among studies, comparison of data becomes quite challenging. Also, often different studies implement different insect harvesting strategies and times (i.e. end of experiment). For example, some studies might use the 'appearance of first pupae/adult' as factor to terminate the experiment while others might use a fixed time (e.g. 20 days) or stagnation phase of the growth curve. In addition, some studies exclude a control treatment, which not necessarily decreases the value of the study, however, it might limit the relevance to other studies i.e. comparison.
This was also found by Bosch, et al. [114]. In this publication the authors expressed the need for guidelines on the standardization of black soldier fly feed experiments and created the first base of a protocol for black soldier fly research.

It is clear that there is a great need for robust guidelines and protocols for insect experimental designs and analytical techniques, especially because many research questions still need to be addressed in order to optimise the production and use of insects. Consequently, insect production and processing will become, most likely, an important field of study worldwide. Therefore, it is now the time to set up initiatives to construct procedures & implement **guidelines** on insect research. This is a very important step in order to be able to compare data, join efforts for conducting research and, consequently, promoting the growth of the upcoming insect industry.

In response to this 'gap', the ValuSect consortium has been working on a policy brief to create awareness on this topic and to make suggestions on research guidelines and protocols. Furthermore, a workgroup from the EAAP congress has already been established earlier for the rearing of BSFL and yellow mealworm for research purposes (feed experiments), of which some of the partners of the ValuSect consortium are part.

In addition, the majority of insect research has been performed on small/laboratory scale. However, this cannot always provide reliable data for larger scale facilities. More **research** should be performed **on realistic/industrial scale** in order to build a bridge between research and practice.

Black soldier fly emissions

From the WP1 literature review performed earlier in the ValuSect project, it was clear that data on insect emissions was still lacking. According to our experience, this topic has an urgency to be addressed as this is not only relevant for breeders to provide themselves with correct infrastructure and safety measures, but also for legislative and licensing authorities. Therefore, emissions related to black soldier fly rearing and measuring methods were included in this literature review. This provides knowledge and information in order to conduct relevant experiments during WP5.

From the literature search it became clear that rearing black soldier fly larvae is accompanied with various emissions and that the kind of emissions is strongly linked to the nature of the rearing substrate. Especially ammonia and methane emissions have been quantified in various studies and these emissions can be considerable when manure was used as a rearing substrate. However, plant-based byproducts from the agri-food chain are not emission free and other factors such as pH, aeration rate or potential overabundance of proteins will be codetermining on how much and which type of emissions will be produced. Among potential emissions the least quantified and hardest to quantify seems to be odour associated with black soldier fly rearing. This should hardly be a surprise as odour is a complex mixture of various compounds that is perceived differently depending on the observer. Some tools such as dynamic olfactometry are available in other industries and should be used in black soldier fly rearing as well to fill in this knowledge gap.

Black soldier fly production automation

As mass production of insects is still in its infancy, machinery and technology that enable the automated production of insects is limited. Consequently, the process currently involves manual labour of many rearing activities, increasing the costs of the production process and therefore end product. Furthermore, the lack on automation withholds the possibility of scaling up the process [23,24,26].

Some insect production facilities today are developing or have developed some technologies for automating the production process, however, they are often not keen on sharing information. Some engineering companies are currently developing software and machinery but often they do not have the practical information and experience to develop relevant products, and/or, are waiting for a market. These products are large investments, which small-scale facilities are often not able to make. Furthermore, development of technology, software and machineries is a long process as this requires research and understanding of insect needs and behaviour.

As not much relevant information is available regarding automation of the insect production process, ValuSect partners involved in WP5 have identified the most labour intensive activities during black soldier fly production. This information can be used by companies to develop relevant technology and machineries. Labour-intensive activities include larvae crate preparation, cleaning, density determination of neonates, harvest of larvae and eggs and feed preparing.

Conclusion & next steps

Through this literature review, partners of WP5 of the ValuSect project aimed to identify knowledge gaps on black soldier fly production. In a next step, an initiation will be made on filling some of these gaps. The knowledge gained will be transferred to the insect for feed sector.

As the rearing of BSF on side streams has already been widely explored, this will not be the focus of the WP5 experiments. Focus will be laid on BSF emissions as in our experience this is an urgent matter to be explored. Furthermore, knowledge on the measurement of emissions has already been gathered in WP1 for yellow mealworm, house cricket and migratory locust. We now aim on transferring this knowledge to insects for feed, i.e. the black soldier fly.

Through this literature review three low-value biomasses have been identified as suitable substrate for black soldier fly (based on insect growth and development parameters), namely catering waste, poultry manure and brewery by-product (beer draff). As these wastes are not similar to each other, they are expected to have different properties regarding emissions and odour. Especially chicken manure is expected to be an extreme regarding odour and therefore considered interesting for emissions measurement. Therefore it is suggested to measure emissions related to BSF rearing on these diets. Data will be compared to conventional black soldier fly diets, i.e. Gainesville diet and chicken feed.

Emissions relevant to measure for BSF and substrates:

- Carbon dioxide
- Ammonia
- Odour (if practically feasible)
- Methane (if practically feasible)

Overview of the actions to be performed in WP5:

WP5 actions		
1. Define experimental design		
2. Optimise measuring methods and provide correct equipment		
3. Transform accumulation chambre(s) of WP1 to be suitable for BSF		
4. Contact side stream suppliers		
5. Emission measurements:	Emission	Method
• Gainesville diet	Carbon dioxide	Sensor
• Chicken feed (farmer crumb/start mash)	Ammonia	Sensor
• Catering waste (Swill)	Odour*	To be defined
• Chicken manure	Methane*	To be defined
• Beer draff		
6. Reporting		

* With reservation

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