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PROteINSECT Agri Business Toolkit

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PROteINSECT Agri Business Toolkit

The PROteINSECT Project analysed and assessed the potential of producing protein for feed from fly maggots [house-fly *Musca Domestica* (MD) and *Hermetia illucens*, often referred to as the Black Soldier Fly (BSF)] reared on organic wastes, with particular focus on the use of animal manures.

The Agri Business toolkit is a collection of information gathered during the execution of the PROteINSECT Project, both from sources outside the project, as well as results of PROteINSECT work. The objective of this Toolkit is to give a structured overview of this information.

The rationale for using manure as a substrate

PROteINSECT was funded to assess the potential of manure as a substrate for the production of fly larvae. The question of user acceptability, as well as the risks associated with manure (biological and chemical) make some of the results obtained in the project more future oriented, and not directly applicable to the current market opportunities in the EU. Outside the EU manure is a commonly used as a substrate for raising insects, ranging from small-scale production units in Africa to semi industrial scale in China. Extensive expertise in the use of insects as human food or animal feed is evident in non-European countries, particularly in International Cooperation Partner countries (IPCP) countries, and thus the exchange of information between ICPC and EU partners and the co-ordinated development of improved production systems is recognised as key to the success of the project.

The value of waste and residual flows

The concept underpinning PROteINSECT is the biological reprocessing of organic waste. This concept, in itself, is not new and there are varied methods available to gain value from organic waste, such as anaerobic digestion. The possibility that insects may be exploited for processing waste streams to produce useful products was proposed as long ago as 40 years, primarily as a means of disposing of animal manure and the generation of insects that could be fed to appropriate livestock. Insect larvae are typically rich in protein (40-75% dry weight), rendering them excellent organisms for the extraction of protein from waste materials. Large quantities of organic waste (in the EU 88 million tonnes/yr of biodegradable organic waste (BOW) material (food waste, garden and public parks waste with 40% ending up in landfill sites [1]) are produced annually by agriculture and the food industry in the UK and Europe and many other economies. For example, in China more than 4 billion tonnes of animal waste is produced annually and at least 20% of this waste has no current value or use. The predicted increases in livestock production that are likely to occur over the next 30-40 years will of course be mirrored by increases in waste mass. Whilst some uses are found for these wastes, such as compost and biogas generation, insects have the potential to utilise these wastes as food and effectively convert it into high value materials, including protein. Many of the above wastes are suitable developmental substrates for a number of dipteran fly species, and as such constitute an unexploited resource for the production of protein. In addition the rearing insects also facilitates significant reductions (up to 60% in 10 days) in waste volumes. Potential uses for residual material include compost, fertiliser, soil remediation material, and as a substrate for biogas generation (anaerobic digestion).

[1] http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=4018

1. Legal and Regulatory barriers
2. Rearing of MD and BSF
3. Processing maggots to feed protein
4. Environmental assessment of rearing maggots (available in early 2016)
5. Limiting factors of the commercial potential of flies for feed

The PROteINSECT project partners are:

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Executive Summary

Considerable progress has been made in understanding the importance of protein nutrition (e.g. the significance of amino acid balance and ileal digestibility) for appropriate feed formulation in recent years. The amino acid strengths and weaknesses of different protein feed ingredients, such as the lysine limitation in maize, and methionine and cysteine limitations in soybean, are now well documented. In the light of the ‘livestock revolution’ and the concomitant growing demands of the feed industry for protein much research has already been conducted to try to identify alternative and enhanced sources of protein for animal feeds. However, with additional competing demands upon land-use for the production of crops for biofuel, together with concerns over the safety and regulation of genetically modified crops, the need to find alternative protein sources for animal feed remains paramount. Of the many insects that have been examined, dipteran flies have protein content and amino acid composition that renders them very suitable for use as replacements to traditional plant and fishmeal sources (Newton et al., 2005).

In the European Union the use of insects as a source of protein for animal feed is currently prohibited for animals raised for human consumption, although an initial scientific opinion has been formulated by the European Food Safety Authority (EFSA).

The current lack of robust safety data is holding up progress on the development and discussion of appropriate legislation within Europe.

Consumer acceptance of insect protein in animal feed has not been fully evaluated.

Current production processes are labour intensive and require further development of semi-automated systems.

What PROteINSECT means …

For producers: There are several existing barriers to market entry and most significantly this includes a lack of data on the safe use of insect protein. PROteINSECT continues to address this need for additional data.

For feed manufacturers: a potential new source of protein for inclusion in animal feed that can be more sustainable and locally sourced, thus reducing dependence (in Europe, for instance) on imported protein crops.

For consumers: Consumer surveys undertaken by PROteINSECT have demonstrated a high level of support for insects in animal feed but there is a need for more information to be made available to enable consumers to make an informed decision.

UPDATE: published EFSA Opinion 8. October 2015

There is still no legal basis to allow insects into the feed chain. The opinion notes the knowledge gaps and uncertainty related to possible hazards when insects are used as food and feed and concludes that there are no systematically collected data on animal and human consumption of insects. Further research for better assessment of microbiological and chemical risks from insects as food and feed including studies on the occurrence of hazards when using particular substrates, like food waste and manure is recommended.

Optimising feed production

The PROteINSECT project is undertaking the co-ordinated development and optimisation of fly production methods for animal feed production in EU and International Cooperation Partner countries (ICPC). Insect rearing systems have been established and existing systems modified using the expertise of project partners.

The systems that have been set up or modified are *Musca domestica* in the UK; *Hermetia illucens* and *M. domestica* in Ghana; *Musca domestica* and *Hermetia illucens* in Mali; and *Musca domestica* and *Chrysomya megacephala* at two sites in China.

The systems range from those suited to mass production at a semi-commercial scale, to systems designed for use by farmers to provide a feed source for their own livestock. Optimisation of all trial systems is ongoing. Importantly, the trial systems have also been used to supply larvae for analysis of quality and safety.

The PROteINSECT project partners have examined the technical specifications, insect yield, inputs, energy use, residual flows, economic costs, productivity and other factors to provide data for preliminary economic and environmental life cycle assessments. The insect production processes within the project have been assessed in terms of the economic and environmental impact to provide recommendations for future research activities. The project has also compiled a set of trial scenarios that will be used to derive recommendations for optimisation and future research.

Despite the potential value of insects as a complementary food source for animals and fish, there are several barriers to market entry that PROteINSECT is working to address:

- The current lack of robust safety data that is holding up progress on the development and discussion of appropriate legislation within Europe.

- Additional nutritional quality data is required to show the potential of the use of insect protein for feed and added value products.

- Consumer acceptance of insect protein in animal feed has not been fully evaluated.

- The current production processes are labour intensive and require further development of semi-automated systems.

Although a number of obstacles remain to commercial advancement in Europe, growing appetites for meat and fish suggest that feed protein derived from farmed insects has huge potential.

The value of livestock production in the 28 EU member states amounted to €169.5 billion in 2013, accounting for 41% of the overall €415.5 billion in agricultural output (FEFAC, 2013). Purchases of compound feed have risen significantly, reaching €55 billion in 2012 (FEFAC, 2012).

Aquaculture production by the EU reached 1.108 million tonnes and a value of €3.365 billion in 2012. Of the main aquaculture species produced, in weight terms the carnivorous Atlantic salmon and Rainbow trout accounted for 14% and 11%, respectively. The most important costs of the EU salmon aquaculture sector are the feed costs, which represented 42% of the total costs in the combined segment and 27% of the costs in the cages segment in 2012 (EU Aquiculture Report, 2014).

The global population is estimated to grow from 7 billion today to 9 billion by 2050 (United Nations, 2009). The historic trend of dramatic rises in meat and fish consumption associated with income growth, increasing urbanisation and lifestyle changes are projected to continue in the decades ahead. The demand for feed to maintain this meat consumption growth is demonstrated by coarse grain production (predominantly used for feed) which is projected to grow by 20% by 2023 (CAP2020, July 2014).

1. Legislation and Regulation

Ambiguous and restrictive European laws concerning the use of insects in feed and food are – for the moment - a major barrier to potential investors and market entry for insect-derived protein. In order to support and encourage the development of industrial-scale insect-rearing plants, there is a need to review current legislation.

PROteINSECT undertook a review of existing legislation and regulation relevant to the use of PAP (processed animal protein) from insects in animal feed, primarily from a European perspective but also in Ghana, Mali, and China. The report 'PROteINSECT Mapping Exercise Report Legislation & Regulation: Europe and Africa & China' is available to download here (http://www.proteinsect.eu/index.php?id=37.)

Current European Union legislation places restrictions on what can go into animal feed:

EU Regulation 999/2001 prohibits all processed animal protein - with the exception of hydrolysed proteins and in some cases fishmeal - being used in animal feed. A recent amendment to this legislation (EU Regulation 56/2013) allows the use of non-ruminant processed animal protein (PAP) in fish feed.

EU Directive 2002/32 seeks to limit undesirable substances in animal feed, covering a range of contaminants and residues including heavy metals, pesticides, veterinary medicines, and environmental pollutants.

The potential for insects to bio-accumulate chemical substances and pathogens present in waste streams has yet to be explored to the standards required to fulfil regulatory requirements for the use of insects as food or feed, raising significant concerns about the safe use of insects in the food chain.

Within the Catalogue of Feed Materials (EC 68/2013), there is no specific entry for 'insect meal' although there is a listing for 'whole or parts of terrestrial invertebrates' suggesting that the use of insect protein in animal feed may be possible. However, it is unlikely that insects will be permitted in Europe for animal feed until thorough consideration of the safety of their use has been made and diagnostic methods for the detection of processed insect protein in animal feed are available to ensure species origin detection.

PROteINSECT has highlighted several regulatory concerns that need to be addressed before the large-scale production of insect protein for animal feed and food can take place in Europe:

- Although PAP is currently permitted in aquaculture feed and is likely to be permitted in pig and poultry feed in the future, these legislative changes do not apply to insect protein. Therefore, legislation that specifically addresses the use of insect PAP in animal feed needs to be developed, provided that it is demonstrated to be safe. Encouragingly, this is a topic that is under discussion at a European level.
- Following a thorough safety analysis (including the safety of those employed in the industry), consideration should be given to adjusting current legislation to permit the rearing of insects on organic waste substances such as manure. This would reduce costs and facilitate a significant reduction in waste volume.
- It is necessary to address new issues that will accompany the mass production of insect protein and implement the appropriate regulatory measures. Specifically the associated environmental impact and animal welfare concerns should be taken into account.
- Clarification of the status of insects as a novel food is required so that a consistent approach can be taken across the EU with regards to placing insects on the market for human consumption. It is believed that the forthcoming novel food regulation (COM (2007) 872 final) will address the current ambiguities.
- Methods for the detection of Insects in the final feed product

Discussions are currently underway within the Safety of the Food Chain Committee of European Commission Health and Consumers Directorate Central (DG SANCO) to change Regulation 999/2001 to allow the feeding of insect protein to non-ruminant animals.

In May 2014 DG SANCO asked the European Food Safety Authority (EFSA) to assess the microbiological, chemical and environmental risks arising from production and consumption of insects as food and feed. In addition EFSA was asked to evaluate the risks posed by the use of insects in food and feed, relative to such risks posed by the use of other proteins sources used in food or feed.

The species covered by the EFSA assessment are:

- *Musca domestica:* Common housefly
- *Hermetia illucens:* Black soldier fly
- *Tenebrio molitor:* Mealworm
- *Zophobas atratus:* Giant mealworm
- *Alphitobus diaperinus:* Lesser mealworm
- *Galleria mellonella:* Greater wax moth
- *Achroia grisella:* Lesser wax moth
- *Bombyx mori:* Silkworm
- *Acheta domesticus:* House cricket
- *Gryllodes sigillatus:* Banded cricket
- *Locusta migratora migratorioides:* African migratory locust
- *Schistocerca Americana:* American grasshopper

EFSA has established a working group of internal and external experts to consider DG SANCO's requests. If they reach a favourable scientific opinion and amendments to the legislation are proposed, this will be a positive enabling step to the production and use of insect protein as animal feed. Still, other areas of legislation will need to be changed. Regulation and standards on the methods of production also need to be established. Results are expected mid October 2015.

Insects and the law

In the European Union, the use of insects as a source of protein for animal feed is currently prohibited for animals raised for human consumption under regulation EC 999/2001.

A recent amendment to this legislation (EU Regulation 56/2013) allows the use of non-ruminant processed animal protein (PAP) in fish feed (but currently not from insect protein)

Under the current regulations, it would not be possible to rear flies on manure or catering waste.

It is unlikely that insects will be permitted in Europe for animal feed until thorough consideration of the safety of their use has been made and diagnostic methods for the detection of processed insect protein in animal feed are available to ensure species origin detection.

UPDATE: EFSA Opinion just published!

The EFSA risk profile document confirms the important role of generating knowledge and data on the quality and safety of the use of insect protein in animal feed.

The primary summary conclusions of the EFSA opinion are:

- when currently allowed feed materials are used to feed insects, the possible occurrence of any microbiological hazards are expected to be comparable to other sources of protein of animal origin and should not pose any additional risk compared to other feed
- the use of other (currently forbidden) substrates to feed insects destined for animal feed such as organic wastes (food waste and manures) must be specifically evaluated.

On-going research and publications in the scientific literature will further the understanding of the safe use of insect protein in animal feed raised on a variety of substrates, including organic wastes such as manure.

	Description	Bottlenecks and critical steps	External links and References	
EU Regulation 999/2001	In the European Union, the use of insects as a source of protein for animal feed is currently prohibited for animals raised for human consumption	The feed ban is the basic preventive measure laid down against TSE and consists of a ban on the use of processed animal protein (PAP) in feed for farmed animals.	http://ec.europa.eu/food/fs/bse/legislation_en.html http://ec.europa.eu/food/food/biosafety/tse_bse/feed_ban_en.htm	
EU Regulation 56/2013	A recent amendment to this allows the use of non-ruminant processed animal protein (PAP) in fish feed (but currently not from insect protein)	The current amendment only allows for protein from animals slaughtered in a certified slaughterhouse. This does not exist for insects.	http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:021:0003:0016:EN:PDF	
EC Regulation 767/2009	It would not be possible to rear flies on manure or catering waste.	Current legislation is firm on the exclusion of manure as a substrate.	http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:229:0001:0028:EN:PDF	
Commission Regulation (EC) No 51/2013	In the EU 2 methods are allowed to confirm the presence or absence of PAPs 1. light microscopy identifies traces of meat and bone meal 2. polymerase chain reaction (PCR) detects the species origin of the meat and bone meal	To ensure that PAP originates exclusively from non-ruminants, according to the current legislation feed has to be tested with PCR; the official control method is criticised because of a number of "low level" positive ruminant results in aqua-feed which did not contain ruminant PAP. Highly specific tests for insect protein in the feed matrix are still under development.	http://www.allaboutfeed.net http://faolex.fao.org/ https://www.wageningenur.nl/	
Overview document		The *International Platform of Insects for Food and Feed* (IPIFF) provided an overview document on relevant legislation concerning the use of insects as food and feed.	http://www.protix.eu/legislation-reports-background-ipiff/	
EFSA Opinion			http://www.efsa.europa.eu/en/efsajournal/pub/4257	

Why are insects not allowed in animal feed?

This whitepaper contains valuable information on the advantages and disadvantages of insects in animal feed. It also gives you a clear view on the current legislations and the changes that need to be made. Why are insects not allowed in animal feed? http://www.allaboutfeed.net/Whitepapers/insects-in-feed/

2. Insect production

The PROteINSECT project has developed and improved production systems for *Musca domestica* (MD) and black soldier fly (BSF).

Fly production is described in stages, with details provided on important and critical steps and or conditions necessary to produce a regular and optimised yield of fly maggots. External links point to guides, informative videos, know how and other information that is freely available. Where applicable known patents have been referenced. A "flow chart" of a sample production (on which an LCA was based) is available in section 3.

Black Soldier Fly (BSF) [*Hermetia illucens*]

A very detailed description of BSF rearing can be found here:

Download : Technical handbook of domestication and production of diptera Black Soldier Fly (BSF) *Hermetia illucens* , Stratiomyidae.

Stage	Description	Bottlenecks and critical steps	External links and References
Adult rearing for egg production	Adults are kept in captivity: large cages. Only water is provided (flies don't feed) using a water dispenser (i.e. wet sponge) and / or sprayed droplets. Optimal parameters: 25±5 ºC; 60±10 % RH. Light (preferably direct sunlight) is essential for BSF mating and eggs production. Artificial light can suits – minimal intensity = $500W/m^2$	Minimum height of the cage is 1.5 m (allow prenuptial flight). Reproduction is possible in small cages (i.ie lab plastic cages 40*40*40cm) provided that ALL abiotic factors are within the optimal range (Temperature, humidity, light intensity). Outdoor production system can opt for a large cage / greenhouse partially covered to offer shelter in case of rain. Optimal density = 5200 flies / m^3 to allow optimal conditions for survival and productivity.	Tom2001 Gob2012 Patents: P_Lee2011 P_Ent2015 Video: V_Lig

Oviposition	Depending on the flies' density and the size of the cage, oviposition devices are made available for eggs deposition. Oviposition devices consist in general of a layer of attractant substrate (manure, fermented or decomposing organic material / wastes) topped with a dry material offering numerous crevices (cardboard strips for example	Natural Oviposition (waiting for "wild" flies to lay eggs on the substrate) is not a feasible approach for high volume production: other insects colonize the substrate before the BSF and production in captivity allows better control. Light is essential (increase intensity = increase eggs production). Gravid females are attracted by the nutritious substrate (odour) ensuring survival for the offspring. Female oviposit on DRY material placed above the substrate (avoid contact). Eggs are 'hidden' in crevices/flutes. Eggs are sensible to moisture: environment must remain humid (thanks to the moist substrate), but direct contact with water cause death.	Boo1984 Gob2012 Car2012
Egg collection	Eggs can be collected every 2 to 3 days. Egg collection consists of collecting the oviposition devices and estimating the amount of eggs collected.	Here, different methods exist for the estimation of the amount of eggs collected/produced: - egg masses can be all retrieved and removed from original support and weighted; - oviposition support is a standard apparatus (can be washed and re-used) whose original weight is known; by difference with the weight post-oviposition eggs weight is determined Frequency of egg collection depends on the farming practises and objectives: more frequent egg collection aims at more homogenous production batches.	Tom2002 Patent: P_Egg2014
Nursery phase	Eggs are placed in a small hatching vessel for 6-7 days during which time they hatch and the small larvae start growing (=seed larvae).	Production step not compulsory but worthwhile for several reasons:	Car2012 Video: V_Nur

	Nursery vessel is a container (box, bowl, etc.) filled with a 3-5 cm substrate layer and covered with a gauze to prevent invasion by competitors if necessary	- It provides optimal conditions for egg hatching and for the larvae to start their development; - It reduces the chances for other fly species to colonise the larval feeding substrate during the first days of development; - It reduces the occupation time of the rearing trays Eggs are highly sensible to humidity and direct contact with water must be avoided	
Larvae production	Post-nursery, seed larvae are added to a culture tray / digester filled with moist nutritious substrate. Larvae will feed and grow in these structures for several days	Several designs exist to grow maggots: from the plastic tray to the concrete bay (digester), choice depends on farming practises and technology available. The substrate use to grow the maggot must be organic. Contaminated material (AB, heavy metals…) can cause high mortality or food safety issues due to accumulation in the produced biomass. Currently in the EU the use of manure leads to regulatory problems: see EC Regulation 767/2009). Several substrates can be mixed together in order to increase nutritional value. Substrate layer should not be higher than 10 cm (max); as the lower part might become anoxic Humidity content of the feeding substrate must be around 60-80% for an easy access to nutrients. If necessary water can be added Depending on the substrate and on the objectives of production, larvae can be fed for 8 to 12 days (production of white larvae), after which they turn into prepupae (mouthparts turn into a hook)	Car2012 Die2009 Biopod Dipt Zac Videos: V_dig V_bin V_Agp Patents: P_Oli P_tra P_New P_Foo

			P Kee
Separation maggot / substrate (harvest)	When the larvae have reached the desired size, they are separated from the feeding substrate Ideally mechanical separation is advised. Depending on the stage of development at harvest, stimuli (heat, moisture level) can force the larvae to leave the substrate.	Technology used by industry are usually kept secret. If the objective of production is to collect larvae (before prepupae, chitin content reduced), the extraction must be forced : - Heating bottom trays (hot water pipes), larvae crawl out using side ramps (30°); - Increase water content of the substrate), larvae crawl out using side ramps (but substrate then needs to be dried); - Passive sieving: tray contents are poured on a sieve placed under strong light, photophobic larvae pass through the sieve. If the objective is to collect prepupae, self-harvest method can be applied: prepupae crawl out (natural behaviour) from substrates using side ramps (30°) on trays.	Dipt Biopod Patents: P_Hem P_Hfl
Pupae production	Post-nursery, seed larvae are added to a culture tray / digester filled with moist nutritious substrate. Larvae will feed and grow in these structures for several days until they reach the prepupae stage and self-harvest Prepupae can be placed in sawdust to pupate (±10 days) Fully formed pupae can be placed directly in a cage for the adult fly to emerge.	Rearing trays / digesters designs can be slightly different from the structure used for larvae production: there should be a 30° ramp for the prepupae to leave the substrate when ready (natural behaviour). Prepupae are gathered in a dry collector. Pupae can be separated from the feed residues by mechanical sieving. Pupae must be kept in conditions of low humidity. Maggots intended to become pupae must be fed properly to store reserve for metamorphosis and the adult stage (feed shortage or malnutrition may reduce the productivity).	ESR Gob2012 Bra1984

		Parasitoids are natural enemies of the BSF pupae. In the geographic areas where BSF parasitoid species have been reported particular care should be provided. To maintain consistent production, at least 10 % of the larvae of each batch should be kept to renew the broodstock.	
Processing	Live larvae / prepupae can be fed directly to farmed animals (fish, chicken). Processing methods can include slaughter (freezing, boiling, drying), defatting if necessary (mechanic press or chemical extraction), drying and grinding (maggot meal). Drying methods available are the same as the ones used in the food industry: sun-drying, convection, forced air or freeze drying.	Drying methods should consider temperatures below 120° C to prevent protein denaturation. BSF larvae are large insects (moisture content = 70%), dewatering may require significant energy. Drying must be optimal to improve the shelf life of the final product (<10% moisture). After 9 weeks storage, dry insect meal may be subject to lipid oxidation and a reduction in quality.	Dry_Dipt FAO Awo2004 (Morakinyo, 2000)
Use of residue substrate	The residues of process are made of a mix of substrate remains and frass (insect excreta) Maggots reduce odour, water and nutrient content and volume of the substrates turning them into valuable bio-fertilizer	Substrate reduction in weight varies from 40 to 75% (dry weight) depending on the substrate	New2005 Yo2009 Alv2012
Other by-products	Chitin can be purified from insect cuticle (exuviae, larvae cuticle) and used in cosmetics, pharmaceutics, water treatment, food industry etc. Fat extracted can lead to biodiesel production	Separation and purification of the main components result in a better economic and nutritional valorisation of the BSF	Li2011 Zhe2012 Fin2007

Infrastructures	BSF production can be managed through different methods. Small scale structure includes biopods® wild females lay eggs inside, larvae grow onto added substrate and self-harvest via ramps and pipes (prepupae) Large scale systems usually include 2 separate units: one for the maintenance of the breeding colonies (cages) and one for the growth of the larvae (trays, bays, digesters, etc.)	Separation of the breeding and the grow-out units is preferable to avoid anarchical egg deposition and to control production (batches)	Car2012 Videos: LFW Ind

Other links:

RedWorm (USA, Videos)

Black Soldier Fly Production with Karl Warkomski (Videos)

Black Soldier Fly Farming (Blog / forum)

Black Soldier Fly blog (Blog / forum)

Technical handbook of domestication and production of diptera Black Soldier Fly (BSF) *Hermetia illucens* , Stratiomyidae.

Musca Domestica (MD)

Stage	Description	Bottlenecks and critical steps	External links
General methodology and infrastructure	Two options can be used for producing house fly larvae: Natural oviposition (NO) consists of exposing substrates to attract naturally occurring house fly females that will lay eggs in the substrate. This can be done, e.g., by building a series of rearing beds 15-20 cm in height on the ground and covered by a roof. A low wall should be built around the beds to protect from strong winds. Adult rearing (AR) consists of rearing adult flies in captivity to produce eggs that will be inoculated in a larval rearing substrate. This requires two separate units: one for the maintenance of breeding colonies (cages or rearing rooms) and one for the growth of larvae (trays, rearing boxes, etc.)	NO is easy and requires limited investment, except for a large ground surface area. Disadvantages are: (1) it can only be done in areas with a high naturally occurring population of flies throughout the year (tropics); (2) it can produce annoyance in the direct vicinity of the production area; (3) other fly species will be present in addition to the house fly; (4) production fluctuates with season; it requires a large ground surface area, although low shelves can be used to increase production capacity per unit area. AR allows the production of larvae in all climatic regions and high numbers can be produced on a relatively small surface area. However, producing high amounts of eggs at low cost can be challenging and is not easy to achieve in basic room conditions. It not only needs good oviposition conditions for adults, but also requires a separate production system for producing adults with high fitness. Critical steps are mentioned below.	Kenis et al. (2014) Čičková et al (2012) Sawicki, 1964 Patent US20120132140 Patent 20120135120 Patent US20040089241 A1
Natural oviposition system (NO)	Substrates are exposed to natural oviposition, e.g. in concrete rearing beds on the ground, in large trays, etc. Substrates should not be exposed for more than 6-8 hours to avoid overcrowding and overlapping developmental	In order for the natural oviposition system to function, the climate and environment must favour the abundance of naturally occurring flies. For example, below 15°C and above 40°C, climates	Nzamujo 1999 Bouafou 2006 Mangunga Ekonda, 2013

	stages. After the oviposition period, the substrate is covered for 2-3 days with a ventilated plastic sheet or fabrics, to prevent further oviposition and to provide a warm environment for larval development. Mature larvae can be harvested in as short a period as three days after oviposition, depending on the climate.	become unfavourable. Beside this limitation, the most critical step is the choice of a suitable and productive substrate. Various substrates can be used, but they need to be both attractive for female flies and suitable for larval rearing. The most suitable substrates include various animal manures (the best are poultry and pig manure), which can be improved by the addition of blood, fish offal or any other highly proteinaceous waste. These substrates can also be used to improve less suitable manure such as ruminant manure. Other organic waste / agroindustrial products such as rumen content, brewery waste, etc. can be used. Easy accessibility and low costs are key for the choice of a substrate. Water content is also essential. Finding the correct moisture content for a substrate requires some trials. Predators can also hamper larvae production, in particular ants, lizards, birds, etc. Protecting the production area with a water canal will help for most predators.	
Adult rearing (AR)	*Musca domestica* reproduce at a high rate relative to other species of flies. Adult houseflies are kept in captivity in room or cages at ambient or controlled temperature. Optimal conditions are 25±5 ºC; 60±10 % RH. Depending on the conditions, females start laying eggs 2-5 days after emergence and, although they can live for up to 1-2 months, the oviposition rate quickly decreases after 10-12 days. Adults are typically fed with a mixture of 50% milk powder (w/w) and 50% sugar (w/w). Water must be constantly	Adults are easy to rear, but the bottleneck in an adult rearing system is the oviposition rate. See row below	Čičková et al (2012) Pastor et al (2011) Makkar et al, 2014 In Chinese: ZL 95117851.2

	available and can be provided using a water dispenser and a wet sponge or fabric. An optimal density for high survival and egg production is around 10- 20000 flies / m^3		
Egg production in AR	Female flies can be very selective about where they oviposit with preferences for particular substrates. Oviposition devices are made available for egg deposition. It can be any attractant substrate (moist animal manure, fermented or decomposing organic material, milk products, wastes) covered by humidified fabric or cellulose paper, preferably in a moistened environment to avoid desiccation of the eggs and the attractant. The most attractive oviposition substrates are frequently those that are most favourable for larval development. The first oviposition of *M. domestica* usually occurs on the 3rd- 4th day after emergence. Oviposition rates decrease 10-12 days after emergence. Eggs are collected daily and placed on the rearing substrates. Eggs must remain in a humid environment (but not directly in water) throughout the process. Alternatively, if eggs are laid in a suitable substrate for larval rearing (e.g. fermented wheat bran), young larvae, rather than eggs, can be inoculated into the rearing substrate, to avoid egg mortality resulting from manipulation.	Egg production is usually the limiting factor in *M. domestica* production systems. Key factors to achieve high numbers of eggs include: -A good source of protein for females. For example, the addition of poultry eggs will enhance egg laying. -Females are most productive between 4 and 10 days after emergence (this period may vary according to the temperature), it is not advisable to keep flies for too long in the cage -Conditions that are too far from the temperature, humidity and crowding conditions mentioned above will result in a poor oviposition rate. -Various designs for the oviposition cups exist. Some are better than others. The best ones include humid and dark areas, which are preferred by females. -Even the best oviposition rates obtained in cages are usually very far from the potential rate (calculated by multiplying the fecundity of a female with the number of females), due to the fact that only a small number of the females lay eggs in a cage. Thus, there is room for improvement through further research.	Čičková et al (2012) Pastor et al (2011) Bryant & Hall, 1975 Lam, 2010 In Chinese: Patent CN1699405A Patent CN102056494B

Larvae production in AR	Eggs are placed in substrates, which can consist of various organic matters (see above for the natural oviposition), in various types of containers, depending on the production size. The depth of the substrates may vary between substrates but should not be too thick (max. 12-13 cm), otherwise only a part of the substrate layer will be used. The optimum quantity of eggs to be placed in the substrate depends on the substrate and the limiting factor (eggs or substrate), should be calculated for each system. In general 0.3 to 1 g of eggs per kg of dry substrate will be used The hatching time is between 8 and 24 hours depending on temperature. Larval development will take between 2 and 7 days depending on the temperature. Containers need to be protected from predators and competing flies, e.g. by a net cover.	The key factor at this stage is the quality of the substrate. Substrates vary tremendously in quality, and even apparently similar substrates (e.g. chicken manure) may provide very different results depending on their origin, storage, composition, etc. As for the natural oviposition system (see above), manure, in particular chicken manure and pig manure, are suitable, as well as various agro-industrial matters such as brewery waste, oil cake, cotton cake, etc., and animal products such as blood, rumen content or fish offal. Easy accessibility and low cost are key for the choice of a substrate. Water content is also essential.	Weigert et al., 2002 Čičková et al (2012) Patent US6938574B2 Patent EP 0365198 A2
Harvesting the larvae	Harvesting larvae from the substrate can be done by: -various sieves and colander methods. Small quantities can be sieved using hand sieves. The upper layers of substrate can be repeatedly removed at first since the larvae tend to move deeper in the substrates as they develop -Larger quantities should be collected using automatic colanders, on which the larvae and substrates are placed. Larvae do not like light and will move and pass through a mesh or holes and fall in collectors. -anoxia, or oxygen depletion. The rearing trays can be placed in sealed containers or plastic bags. The larvae will move out by themselves and, so, can be easily collected outside the containers.	Extracting larvae is not critical but can be time consuming. The method will depend on the size of the production system and the cost of labour.	Patent US3814057

	Before being used for feeding animals or for being dried, larvae should be purged, i.e. to void the gut contents, which is carried out by leaving the larvae without food for some hours or overnight. To clean the larvae, dry wheat bran or sawdust can be added, which will then be sifted out again.		
Pupae production (AR)	Pupae are needed to produce adults for egg production. Fitness needs to be maximised and, thus, large pupae and adults are needed. Larval rearing containers are left to allow larvae to continue their development until maturity. Mature larvae are then extracted from the substrates and placed in sawdust to pupate. Alternatively, pupae can be extracted using water since they float on the surface. Fully formed pupae are weighed to evaluate their number (1 g = ca. 60 pupae) before being placed directly in a rearing cage or room to obtain adults.	Parasitoid wasps are important natural enemies of pupae. They can seriously lower the emergence rate. Therefore, no matter what containers are used to contain mature larvae and pupae, they have to be protected from parasitoids, e.g. with a fine screen or gauze protection.	Čičková et al (2012) Mann et al., 1990
Processing	Fresh larvae can be directly fed to farmed animals (poultry, fish etc.). In most cases, however, larvae will be kept dry. Several killing and drying methods are available and include freezing, boiling, sun drying, drying in an oven, (microwave, solar dryer, convection, forced air, etc.). Dry larvae can also be ground and, defatted (mechanic press or chemical extraction).	Drying methods should consider a temperature below 100° C to prevent protein denaturation. Drying must be optimal to improve shelf life of the final product (<10% moisture). The killing and drying methods will largely depend on the size of the production system, the region, the use of the larvae, etc.	FAO Patent CN102425918A Patent CN1868298A Patent CN101836686A In Chinese Patent CN101838582A Patent CN1666654 A

Use of residue substrate	The residues of the rearing process are composed of a mix of substrate remains, frass (insect excrement) and dead larvae. Larvae reduce the odour, moisture content, and volume of the substrate without significantly reducing the nutrient content turning them into a valuable bio-fertilizer Substrate reduction in weight varies from 40 to 75% (dry weight) depending on the substrate.		
Other by-products	Chitin can be purified from insect cuticle (exuviae, larvae cuticle) and used in cosmetics, pharmaceutics, water treatment, food industry etc. Extracted fat can be used as biodiesel.		Manzano-Agugliaro, 2012 Patent CN 101880591 Patent CN1258678A Patent CN102796611A

https://www.youtube.com/watch?v=2u3eQnaODzw

3. Insect Processing

Process	description	Bottlenecks and critical steps	External links and References	
Drying	Larvae are dried/dehydrated in: - Warm air (oven) - Open air / sunshine - Alternative technologies Final water content of the larvae is 10 % (w/w).	Avoid over-drying larvae. A humidity of 10 % is needed for ideal complementarity with other feed ingredients. Otherwise, the larvae or other feed ingredients can start hardening, making feed processing difficult. Over-drying can also alter nutrient composition of the larvae as well cause nutritional losses. This way, dry the larvae in thin layers on black surfaces. Sun-drying is only possible when relative air humidity is low. Otherwise, drying is retarded and there is a risk of contamination with pathogens. Use only sun-drying in case of dry air. Alternative technologies are 1/ more expensive, 2/ less sustainable and 3/ need special technical skills. In all cases, you have to respect animal (insect) welfare issues, like using a suitable killing protocol for insects before extraction. So far, freezing and cooking are considered as the most ethical killing methods.	http://www.pjbs.org/pjnonline/fin1187.pdf	

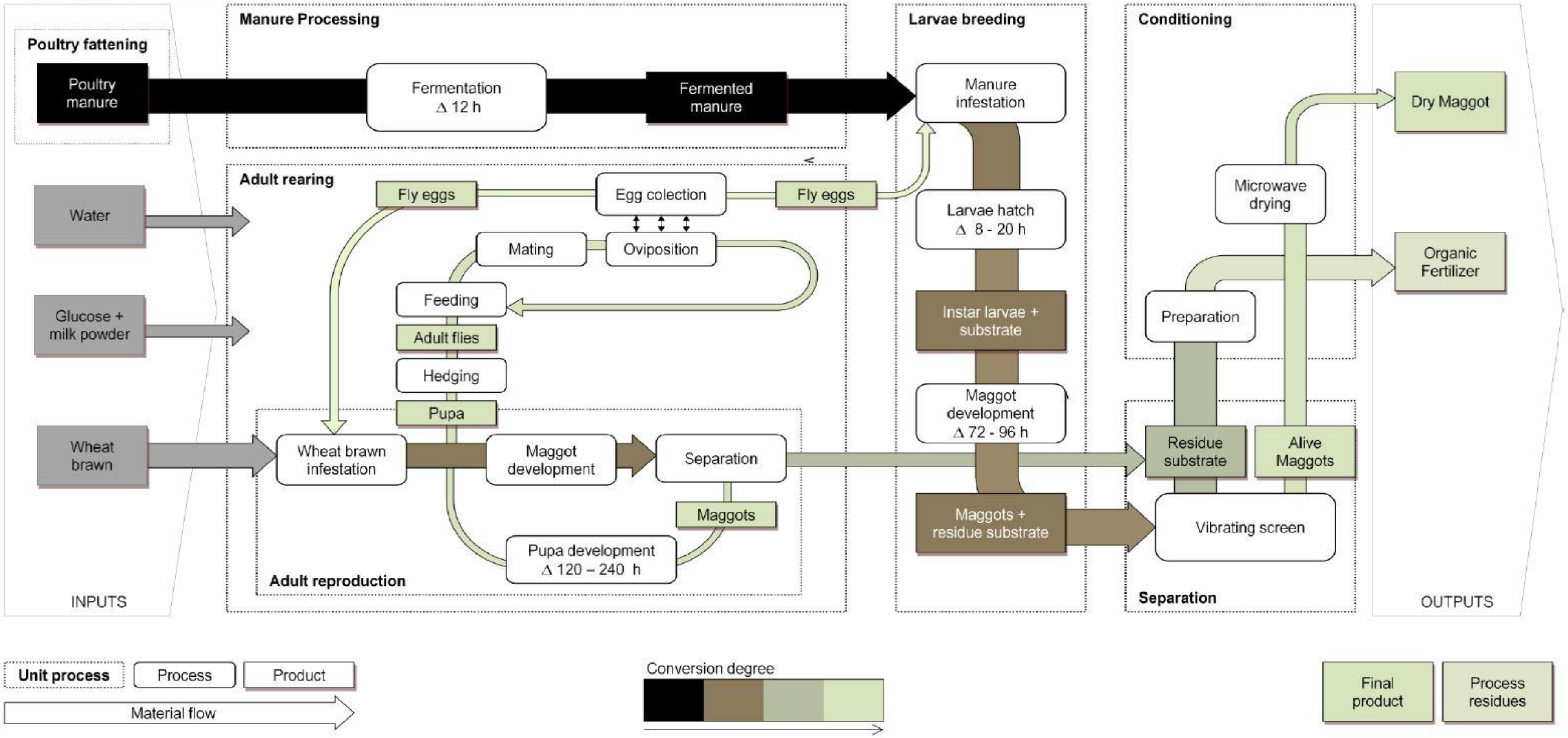
Grinding	Prior to processing larvae can be: - Ground (dry larvae) or - Blended (wet larvae)	Both procedures result in a fatty blend polluting equipment. Fatty residues can be easily recovered using hot water or a feed-grade solvent, such as hexane.		
Protein extraction	Proteins are extracted from larvae biomass by: - Chemical extraction (1/2 w/v ratio) - Physical extraction - Biotechnological processing into single cell protein	Chemical extraction is performed using feed-grade solvents, for example hexane which removes the oil fraction of insects. The residue is enriched in proteins. Take care that all solvent is removed for further processing of the insect protein. Less advised extraction protocols are based on hot water or acid/base. Physical extraction is preferentially performed in oil presses, where oil is separated from proteins via membranes. Salting in or selective adsorption processes are less in favour. Biotechnological processing includes fermentation with feed-grade microorganisms, co-fermenting inorganic nitrogen into organic nitrogen. Fermentation processes are very sensitive to pathogen contamination and need semi-sterile processing conditions. An advantage is that chitin is destroyed during fermentation. For extracted proteins, the presence of parasites on fly larvae, influencing safety of extracted insect protein has to be considered. However, a heat treatment before processing mitigates this issue. In addition, residual substrates in the gastrointestinal tract and on the surface of larvae have to consider legislation in the country of origin. However, fasting and washing insect larvae before processing can mitigate this issue.	https://www.youtube.com/watch?v=XXjds_lBvC4 https://en.wikipedia.org/wiki/Hexane http://lipidlibrary.aocs.org/processing/solventextract/index.htm https://www.youtube.com/watch?v=x1lPFswKfYU https://www.youtube.com/watch?v=FyFFRBYCzkA&list=PLWHg3f1i4hU5-Zl76vOdNKq7SjIaCb6o5&index=24 https://en.wikipedia.org/wiki/Single-cell_protein https://en.wikipedia.org/wiki/Fermentation https://www.youtube.com/watch?v=5eKdZ0dVCCo https://www.youtube.com/watch?v=VKpthcW1llU http://worldwide.espacenet.com/	

Lipid extraction	Lipids are extracted from larvae biomass by solvent extraction (1/2 w/v ratio)	Chemical extraction is performed using feed-grade solvents, for example hexane which removes the oil fraction of insects.	https://www.youtube.com/watch?v=XXjds_lBvC4 https://en.wikipedia.org/wiki/Hexane http://lipidlibrary.aocs.org/processing/solventextract/index.htm	
Chitin extraction	Chitin is extracted from larvae by: - Enzyme treatment - Acid/alkaline treatment	Although proteases are the most promising type of enzyme for extracting intact chitin from insects, its use is not allowed in animal nutrition. Acid/alkaline treatments are the most promising, but too stringent conditions can lead to degradation of chitin or conversion to chitosan. Possible toxicity issues associated with feeding extracted chitin to animals are important. So far, no clear consensus exists on the toxicity of chitin. This way, the final chitin structure and presence in processed proteins needs further attention. Small oligostructures of chitin (like chitosan derivatives) can have interesting features in animal nutrition, while long molecular weight chitin can show anti-nutritional effects. Although the presence of chitin in insect larvae proteins can pose a potential risk in protein processing into animal feed applications, it is likely that the insect protein will be used at relatively low levels in diets and actually can be good for animals as it can promotes a positive immune response.	https://en.wikipedia.org/wiki/Chitin https://en.wikipedia.org/wiki/Chitosan http://www.google.com/patents/US20140100361 Henry et al. (2015). Review on the use of insects in the diet of farmed fish: past and future. Animal Feed Science and Technology, 203, 1-22.	

4. Environmental Impact and Sustainability

The LCA of the various maggot production setting is currently in progress and will be added in early 2016

GEI_System (People's republic of China)
House fly [*Musca dormestica*]



LCA Relevant links and literature:

http://www.biw.kuleuven.be/lbh/lbnl/forecoman/docs/Generic LCA proteinsect.pdf

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0051145

http://lcafood2014.org/papers/26.pdf

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3012052/

http://www.stir.ac.uk/media/schools/naturalscience/aquaculture/aquasect/images/Agritt LCA pres.pdf

5. Commercialisation and its obstacles

The development of alternative and additional protein sources to address the growing global demand for meat and fish is not a new concept. Two important factors - the recognition of the potential value of insects as a complementary food source for animals and fish, and demand for new sources of protein for animal feed - are driving commercial development. Research shows that the by products of rearing of insects for animal feed has additional commercial potential as compost, fertiliser or for the production of energy.

Asia and Africa in particular have developed extensive expertise in the rearing of insects. In Europe, however, ambiguous and restrictive laws concerning the use of insects in feed and food are seen as a major barrier to potential investors and thus market entry for insect-derived protein. Commercial insect rearing in Europe is largely limited to the production of fly larvae for recreational fishing, and crickets and mealworms (the latter on an industrial scale) for pet food.

Insect species considered most suitable for feed production include silkworms, mealworms, black soldier flies and the common house-fly. As fly larvae can be reared on a wide range of wastes, they offer a potential solution to help reduce the increasing quantities of organic waste produced by the agriculture and food industries. The potential use of waste substrates to yield protein rich fly larvae has been demonstrated by the successful establishment of systems with varying production capacities (25-250 kg wet larvae per week) in China, Africa and the UK. Furthermore, the residual material of larval digestion has economic value as a fertiliser or soil conditioner.

The potential exploitation of residual materials is a secondary goal of the PROteINSECT project. PROteINSECT is aiming to ascertain the most economically viable end-use of the materials that remain following digestion by insect larvae. The use of residues as fertiliser is particularly attractive given the recent rises in global prices of chemical fertilisers, with prices rising by more than 50% for particular blends.

Preliminary studies have suggested that residues can be utilised as an agricultural fertiliser. However, the sporadic and localised occurrence of insect production systems has, to date, prevented any systematic evaluation or analyses of the potential values of residual flows. PROteINSECT is advancing the state of the art by carrying out a comprehensive evaluation of residues derived from a number of different insect/substrate combinations under a range of climatic conditions. A combination of chemical analyses and laboratory and field scale studies is enabling the added value of residual flows to be ascertained.

Examples of commercial enterprises

Agriprotein, a South African company established in 2009, is considered the world leader in the mass production of fly larvae. The firm focuses on nutrient recycling using organic wastes to produce insect-based protein feed, extruded oil and fertilisers. House-fly, blow fly and black soldier fly larvae are all 'farmed' in a factory. Its goal is to produce 7 tonnes of insect meal, 3 tonnes of oil and 20 tonnes of fertiliser per day and add 10 production facilities by 2020.

Enterra Feed Corporation in Canada uses food processing and distributor waste to rear black soldier fly. The company produces protein and oil products for aquaculture feed, animal feed and pet food. The digestate from the larvae is processed and sold as fertiliser.

Enviroflight, a U.S. firm, uses co-products from breweries, ethanol production, and pre-consumer wastes to rear black soldier fly larvae. The larvae are processed into meal and sold as feed for carnivorous fish, while digested feedstock is sold as feed for omnivorous fish.

PROteINSECT Partners:

Grantbait (UK) has operated a fish-bait breeding system for more than 20 years. It has a production capacity of 8-10 tonnes of maggots per week. It uses pig offal acquired from an abattoir to nurture maggot development. A new system of *Musca domestica* rearing for animal feed is presently being developed at Grantbait, with support from FERA.

Musca domestica maggots for poultry feed by IER (Mali)

IER in Mali began the production of *M. domestica* maggots for poultry feed at IER in the 1990s but was suspended before being restarted with PROteINSECT. Maggots are used to feed poultry or fish, either fresh or after having been dried in the sun.

GEI (China) has always maintained a laboratory rearing of *M. domestica* for research purposes. However, the data provided here are from a previous, larger scale rearing system that forms the basis of the rearing system presently being developed by GEI.

HZAU (China) has a long expertise in maggot production, in laboratory as well as on farm conditions. Their research has focused on the use of housefly maggots for various purposes, i.e. not only animal feed but also, e.g., para-pharmaceutical products in traditional Chinese medicine. Their main research results are available in the Chinese literature but the most relevant aspects will be published as a review in the framework of PROteINSECT. HZAU is presently developing a new insect production facility at a chicken farm, based on their previous expertise and using the best techniques presently available. The potential to improve the efficiency of all steps of the production process are being investigated within the PROteINSECT project.

Commercial operations exist in countries that include South Africa, Canada and the United States. Companies with similar ambitions have been established in Europe, although they are currently limited by legislation that does not permit the use of insects in livestock feed. In Europe:

- PROtix Biosystems BV in the Netherlands has developed scaleable insect production systems using "end-of-life streams" to produce insect meal and purified oil, as well as chitin as a basis for derivatives like chitosan.
- Bioflytech in Spain specialises in rearing a range of dipteran species producing biomass for animal feed with additional focus on the use of insects in the development of technologies for waste valorisation.
- Biological control companies such as Koppert in the Netherlands and Hermetia in Germany are ideally placed to enter the market owing to their significant expertise in rearing pollinators (including flies) and beneficial insects.

To assist private firms, the International Producers of Insects for Food and Feed (IPIFF) was formed in 2013.

The main obstacle to commercialisation in the EU is the current legislation that not only limits the use of insects in feed, but also excludes the use of manure as a feed substrate. The willingness to change will require solid scientific data on safety for humans and animals. This will enable the ECs decision-making process towards new legislation. The EFSA opinion is favourable towards including insects as a legitimate animal feed, and may even see benefits in the use of manure as a feed substrate. At this time this seems far fetched and extensive safety data would be required to establish if this approach is feasible. However, if "food wastes" become a legal insect substrate, they will presumably also become a legal livestock feed leading to direct competition and presumably an increase in substrate value. It remains to be seen if this would allow insect production on "food wastes" to remain competitive, or if insect farmers will need to fall back on less expensive (or free) substrates, such as manure.

Outside the EU, the use of insects in feed has far fewer challenges. In China, there are small scale industrial applications with fermented manure "up-cycled" on site at chicken farms. The MD maggots are fed live to the chickens (COMMNET Video). Similar scenarios are in Ghana and Mali, where small scale farming (and training) is taking place.

The feeding trials being undertaken by PROteINSECT are still in progress. As the results are made public, they will be added to the toolkit. (forecast to be available early 2016)

	Description	Bottlenecks and critical steps	External links and References
EU/ national Patents	Several maggot farmers are attempting to patent their technologies. In this toolkit, we will not assess the patents limiting characteristics. However, we have come across several patents.	These patents may or may not limit the possibility of implementing certain technologies in the respective countries. Whether or not this is the case will need to be cleared on an individual basis.	In order to get an up-to-date situation of the respective patents, please screen the Open-access Espacenet database with the relevant keywords
		Chitin may act as an anti-nutritional factor (negative effect on gut health (toxic), feed conversion efficiency, etc.) Although the presence of chitin in insect larvae proteins can pose a potential risk in protein processing into animal feed applications, it is given that	http://japr.oxfordjournals.org/content/20/1/1.full

		the insect protein is likely to be used at relatively low levels in diets and actually can be good for animals as it promotes also an immune response. In addition, chitin derivatives such as chitosan, can show antimicrobial characteristics, and act as substitute for antimicrobial growth promoters in animal nutrition.	
		If protein is extracted (with e.g. hexane) from insects some residual solvent may be present in the end product	
		How effective are parasites/pathogens, potentially present in the insect product, killed/destroyed during processing of insects?	
		Rearing and processing will have to deliver a product which is easy to handle and which can compete nutritionally with conventional protein sources	
		What about effectiveness of feeding whole insects (including insect fat) vs insect protein?	
			Whitepaper_Insect_meal http://www.allaboutfeed.net/ http://www.nieuwsblad.be/

			http://www.fao.org http://www.feednavigator.com/
Fish			https://www.animalsciencepublications.org/publications/af/articles/5/2/37
poultry & Pigs	Poultry feed is made by mixing a premix or concentrate (containing all vital small-inclusion ingredients such as vitamins and minerals) with bulk ingredients (soybean meal, cereals, oil, …) into a final feed. Crude insects and/or purified insect proteins are fed to poultry, ideally replacing around 1/3 of the conventional protein source (soybean meal [and/or fish meal in the case of piglets). In case of feeding whole insects, some fat can be exchanged in the formula as well.		http://www.fao.org/docrep/018/i3253e/i3253e07.pdf http://www.feedipedia.org/content/insect-meals-animal-feed http://www.pigprogress.net/Home/General/2014/9/Insects-in-pig-feed-1592213W/

TOOLKIT PROTOCOLS

A. Understanding safety implications of insect larvae for use in feed[2]

The potential for insects to bio-accumulate chemical substances and pathogens present in waste streams has yet to be explored to the standards required to fulfil regulatory requirements for the use of insects as food or feed, raising significant concerns about the safe use of insects in the food chain.

The persistence of chemical residues, such as antibiotics and pesticides through the food chain is of particular concern where, for example, manure or anaerobic digestate is used as feedstock, possibly leading to longer term issues such as antibiotic resistance in livestock. The use of food waste as feedstock generates further concerns over microbiological safety and the formation of natural toxins produced during food spoilage such as mycotoxins. Industrial toxins such as dioxins may also be important depending on insect rearing and preservation processes.

Chemical risk

Analysis undertaken is shown in Figure 1. All methodologies are described in Charlton, et. al., (2015).

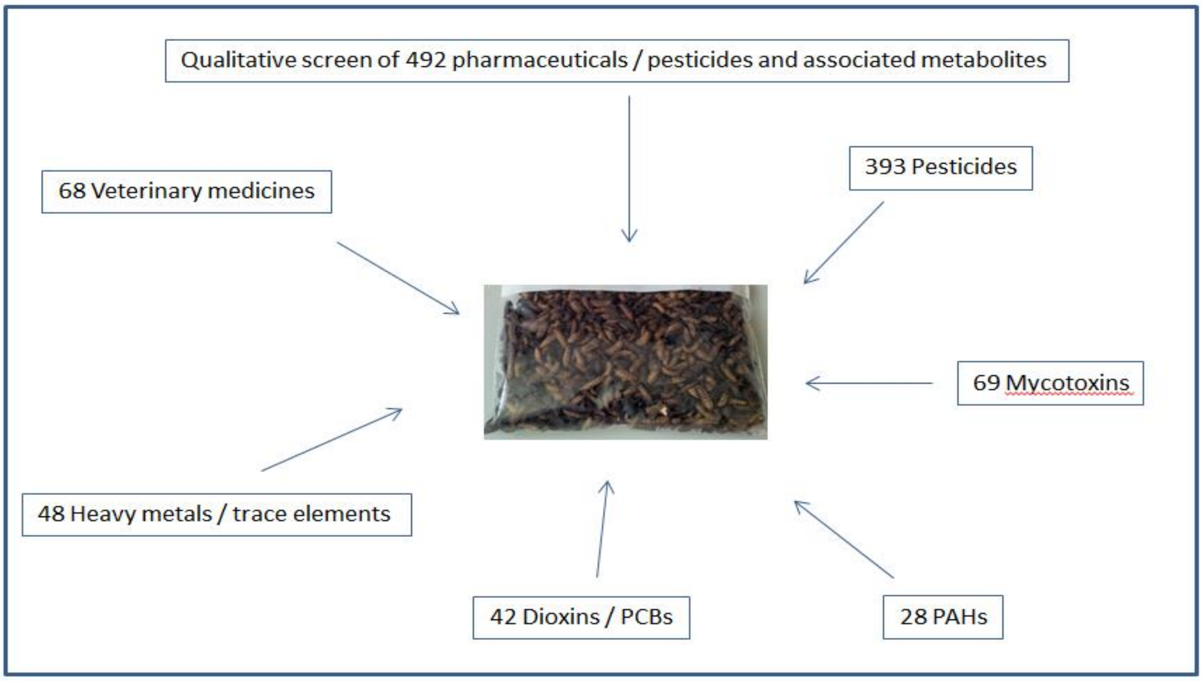


Figure 1. Summary of chemical analysis undertaken on larvae samples.

[2] Text and any data shown in this document are from our paper published in JIFF. Charlton, et al 2015.

Allergenicity

There is currently an unknown risk for livestock of allergenic proteins in insects. Tropomyosin, an allergen responsible for shellfish allergy, is also present in many insect species. Cross-reactivity of insect proteins to crustacean allergic individuals has been demonstrated. Whilst this is clearly important in making choices in relation to entomophagy, it is also a major consideration in relation to insects for use as animal feed as allergenic response in farm animals will result in animal welfare concerns in addition to economic and nutritional implications in relation to, for example, weight gain and meat yield.

Analysis in Proteinsect - Each sample (assumed 100 µg protein) was proteolysed following denaturation, reduction and alkylation of cysteine residues. Analysis of the samples was performed using nanoflow liquid chromatography with high resolution mass spectrometry (HR-MS). Raw data were searched using Proteome Discoverer (v. 1.4, Thermo) in combination with Mascot (v. 2.4.1, Matrix Science) as well as PEAKS (v. 7 demo, Bioinformatics Solutions Inc) for improved identifications using *de novo* based sequencing. Database searching was performed against the SwissProt *Muscomorpha* fasta database containing both reviewed and unreviewed entries. Proteins previously notified as potential allergens in crustaceans; tropomyosin, arginine and myosin light chain were identified.

Microbiology

So far, no major microbial risks are associated with use of insect larvae, except for the indirect contamination by substrates or by cross-contamination during processing. In the first case, a heat treatment (eventually combined with washing of the insect larvae) can help to avoid microbial contamination at later stages. In addition, hygienic processing technologies cope with cross-contamination issues.

TOOLKIT PROTOCOLS

B. Establish nutritional composition of all potential feed products

In animal nutrition, an appropriate available energy and amino acid supply in a balanced diet for efficient protein use by livestock is of critical importance and a high energy to protein ratio is needed to optimize the use of the protein. The quality of the protein is important in animal feed and essential amino acids need to be present for a balanced diet. In addition to protein, total fat and its constituent fatty acids are also an essential component of the diet. Nutritional content of larvae are often variable and depends on the diet on which the larvae have been reared. Figure 1 describes the analytical methods employed to evaluate nutritional content of the larvae.

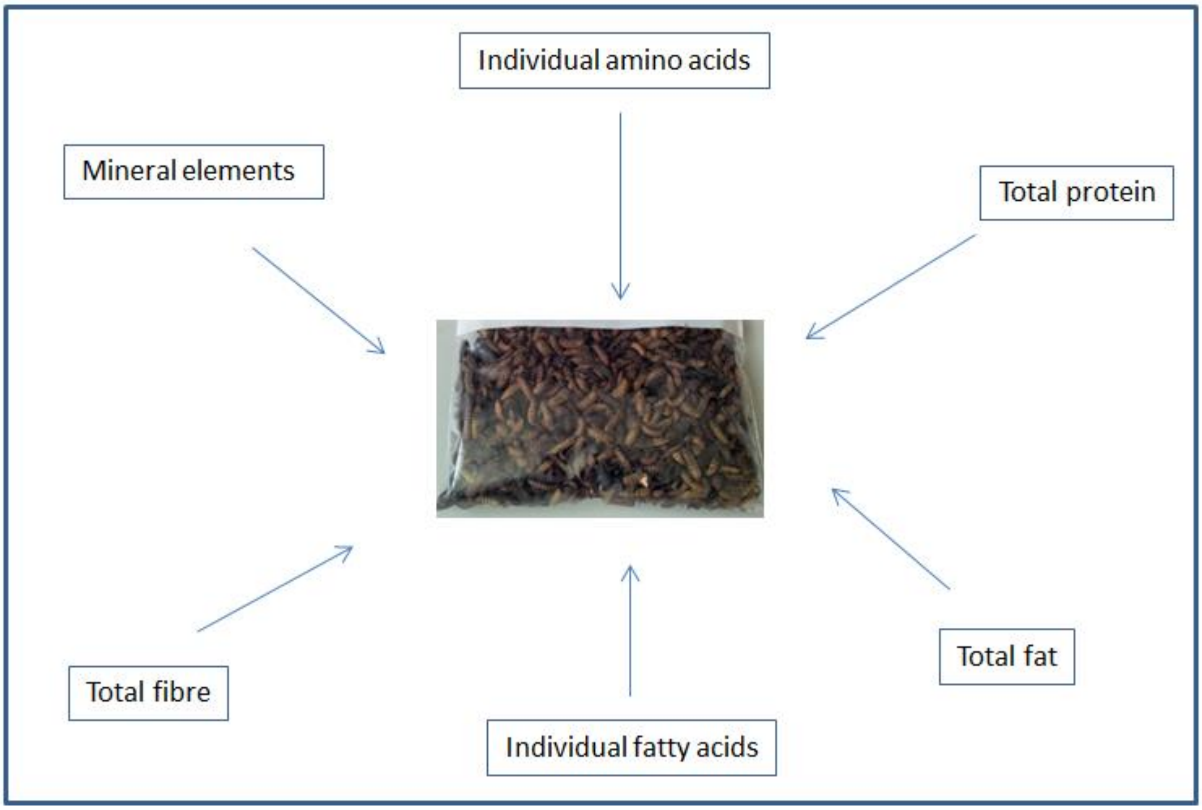


Figure 1. Summary of analytical methods used to evaluate nutritional parameters of insect larvae in Proteinsect.

Analysis in Proteinsect

Total Protein – Total protein was analysed using two separate analytical techniques. Initially total protein via total nitrogen content was determined according to the Kjeldahl method. A more accurate quantification of protein was undertaken using the summed concentrations of all individual amino acids.

Amino acid analysis - Samples were hydrolysed in the presence of strong acid and base under vacuum for 22 hours. After hydrolysis amino acids were determined by LC-UV with a post column derivitisation.

Total Fat - Total fat analysis was conducted according to Commission Regulation (EC) No 152/2099. The sample was extracted with light petroleum. The solvent was distilled off and the residue dried and weighed.

Fatty acid analysis - Fatty acid profiles for each of the substrate types were determined as described by Christie (1993): Preparation of ester derivatives of fatty acids for chromatographic analysis. *Advances in Lipid Methodology* 2:69-111.

Total Fibre – A sample (post fat removal) was acidified, filtered, weighed and ashed at 485°C. The loss of weight resulting from ashing corresponds to the crude fibre present in the test sample.

Minerals and trace elements – Each sample was burned in a furnace before being acidified and diluted. Elements were determined by Inductive Coupled Plasma – Atomic Emission Spectroscopy.