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

Domenico Caruso
Emilie Devic
I Wayan Subamia
Pascale Talamond
Etienne Baras

PT Penerbit IPB Press
Kampus IPB Taman Kencana
Jl. Taman Kencana No. 3, Bogor 16128
Telp. 0251 - 8355 158 E-mail: ipbpress@ymail.com
Penerbit IPB Press @IPBpress

Perikanan
ISBN : 978-979-493-610-8
9 789794 936108

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

IRD Éditions

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

Domenico Caruso
Emilie Devic
I Wayan Subamia
Pascale Talamond
Etienne Baras

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

IRD Institut de recherche pour le développement
isem

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

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

Domenico Caruso
Emilie Devic
I Wayan Subamia
Pascale Talamond
Etienne Baras

IRD Éditions
IPB Press

TECHNICAL HANDBOOK OF DOMESTICATION AND PRODUCTION OF DIPTERA BLACK SOLDIER FLY (BSF) *HERMETIA ILLUCENS*, STRATIOMYIDAE.

Editors:
Domenico Caruso
Emilie Devic
I Wayan Subamia
Pascale Talamond
Etienne Baras

Editor : Dwi Murti Nastiti
Cover Design : Sani Etyarsah
Layout Design : Ardhya Pratama, Bambang Dwi Susilo
Corrector :

PT Penerbit IPB Press
Kampus IPB Taman Kencana Bogor

First Edition: December 2013

Printed by Percetakan IPB

ISBN: 978-979-493-610-8

ABSTRACT

This technical book aims to publish required key information for domestication and production of the insect *Hermetia illucens*. Also known as « Black Soldier Fly » (BSF), this cosmopolitan dipteran, belonging to the family of Stratiomyidae, is considered as non-pest.

Being saprophagous at the larval stage, it is able to biodegrade various organic wastes. For years, recycling of domestic or agricultural wastes has been a continual concern and the potential use of BSF larvae in this field already raised the interest of many researchers. However, the recent awareness of the scarcity of food resources for livestock has led French and Indonesian researchers to consider BSF as a new animal food resource.

This is the core of the Bioconversion Project held in Indonesia which - through the construction of an experimental pilot - allowed the mass production of this insect. This book explains how to produce BSF adults, eggs and larvae for animal –feeding, with a particular emphasis on fishes. Besides methods of production of BSF, this technical handbook summarizes the successive steps of the project in order to specify the potentialities and limits of using BSF larvae for feeding fishes in tropical aquaculture.

A thorough economic analysis stresses both strong and weak points of the pilot and provides elements of decision making for producers. BSF productions were mostly obtained while using Palm Kernel Meal (PKM) as a substrate. However, other substrates have been experimented at different scales to consider alternative productions.

Abstract

This technical book, which was prepared for a broad audience ranging from scientists to producers, sums up knowledge on BSF biology and rearing. So it aims at spreading knowledge and know-how gained during the Bioconversion Project.

Besides, we hope this handbook will stimulate the readers to act for the direct or indirect promotion of insects as new food resources for mankind.

ACKNOWLEDGEMENT

The bioconversion project would have been impossible to achieve without beneficial action of many people. Among them, authors wish to warmly acknowledge Mr. Fatuchri Sukadi, from "Balai Riset Perikanan Budidaya Air Tawar" (BRPBAT) thanks to whom the project started in Indonesia. The experimental pilot in Depok was an expensive research tool. The building and operating costs have been strongly supported by IRD – particularly by the "Direction de la Valorisation au Sud" (DVS) as well as by the "Service de Coopération et d'Action Culturelle" SCAC from French Embassy in Indonesia.

We also wish to acknowledge every student and worker involved on a daily basis in the development of research on BSF. Special thanks go to Mr. Urip who participated to the bioconversion project since its start.

Last but not least, writing this handbook was not a pushover and the authors wish strongly acknowledge all the colleagues from IRD/ ISEM for critical comments on early drafts of this manuscript, namely Marc Legendre, Jacques Slembrouck, Rémi Dugué and Sophie Quérouil-Carles, who have largely contributed to the improvement of this handbook. Special thanks to Dominique Caseau-Baras, for her patient and thorough reviewing of the English style of the manuscript.

CONTENTS

ABSTRACT ..... **v**

ACKNOWLEDGEMENT ..... **vii**

CONTENTS ..... **ix**

INTRODUCTION ..... **xi**

Chapter 1 Biology of *Hermetia illucens* 1

Chapter 2 Presentation of pilot production **11**

Chapter 3 Substrates for rearing **21**

Chapter 4 Rearing of adult BSF and egg production **37**

Chapter 5 Production of pupae **51**

Chapter 6 Rearing of Fattening BSF Larvae **65**

Chapter 7 Ecological Intensification of Aquaculture: Perspectives offered by BSF **77**

Chapter 8 Nutritional characteristics of Hermetia illucens for Fish Farming **89**

Chapter 9 Economic Aspects of Production **101**

CONCLUSION AND PERSPECTIVES **107**

REFERENCES **123**

GLOSSARY **137**

INTRODUCTION

Demographic evolution, impact of climate change on natural resources and necessity of their sustainable management, require increasing the global food production by about 70% before year 2050 in order to feed the 9 billion inhabitants the planet will count by then. The necessity to feed such a growing population generates a constant pressure on both plant and animal productions. This is especially true in South East Asia, which is the most densely populated region in the world. Industrial rearing of chickens, pigs and fish is in continuous expansion due to increased consumption by humans. This growth is intimately dependent on the availability of protein sources for sustaining animal productions.

The production of vegetal protein-rich soybean meal supports this demand to a great extent with a worldwide production of about 124 million tons (United States Department of Agriculture, 2012). However soybean intensive cultivation has a strong impact on environment (deforestation, pollution, GMO) and the sustainability of this production remains widely debated. Fishmeal, with over 4.7 million tons produced yearly, is the main source of animal protein. Animal proteins generally display higher biological value than plant proteins and they are much more used to feed fishes in aquaculture.

Fishmeal essentially originates from the fisheries of small pelagic fishes, mainly Peruvian anchovies. Tacon and Metian (2008) estimated that 68% of this fishmeal was intended to aquaculture food. The use of this resource is rightfully questionable.

Moreover, the stagnation of wild fish captures, linked to an increasing and endless demand for animal rearing purposes, makes this resource increasingly expensive and limited.

The availability of food in aquaculture is restrained, more particularly as regards the sources of protein. Therefore the use of agricultural by-products and unconventional animals as bioconverters becomes a true necessity.

Although rather anecdotal until now, insect rearing opens heuristic perspectives well beyond the field of animal feeding.

According to FAO (2012), no less than 527 species of insects are currently consumed by humans in 78 countries. Most of them are collected in the wild, whereas their rearing in controlled conditions could be much more advantageous for many purposes, including their use as protein sources for other animal productions.

The Black Soldier Fly is often found in the vicinity of poultry and pig rearing units where their larvae feed on manure or compost (Furman *et al.,* 1959; Booth and Sheppard, 1984; Newton *et al.,* 2005b). BSF Larvae are able to utilize massive amounts of organic waste and reduce their unpleasant smells (Lardé, 1990).

They reduce efficiently the accumulation of polluting compounds (nitrogen, phosphorus) from manures and composts. They also modify the bacterial microflora by reducing the ocurrence or abundance of undesirable bacteria (Erickson *et al.,* 2004; Yu *et al.*, 2011).

Therefore, BSF larvae increasingly emerge as a sustainable alternative method for organic waste treatment. Moreover, they are efficient converters as they produce a protein and lipid-rich biomass from substrates that can be poorly used by monogastric animals. These characteristics, linked to a short production cycle, make BSF larvae very good candidates for intensive production, as far as the domestication and the production of the insects are controlled.

Initiated and developed owing to the clear-sightedness of Saurin Hem (French researcher from IRD), the “Bioconversion project”, based on the recycling of Palm Kernel Meal, PKM)′ by BSF, is an international project. Originally conceived in Western Africa this project has been deepened and developed in close cooperation with Indonesian scientists from BPPKP-Depok[1].

A semi-industrial pilot rearing unit that, as far as we know, has no counterpart in the world at present, has been developed to perform extensive experimentations. It also gave the opportunity to study the production of BSF adults, eggs and larvae for animal - especially fishes - feeding.

This internationally shared research would not have been developed without the perseverance of Saurin Hem (IRD) to whom this handbook is largely dedicated.

Saurin was initially concerned with the development of fish farming in small villages in Guinea-Bissau in Western Africa, where resources were limited and other ways of rethinking and developing aquaculture were needed. Owing to his sharp sense of observation, Saurin deepened in studying unidentified larvae he spotted in PKM crop waste (he discovered shortly thereafter these were BSF larvae) and he wisely anticipated this might be an alternative to provide alternative sources of proteins for fish food. Since then his career of oceanographer and expert in aquaculture has been indissolubly linked to the Black Soldier Fly. The first productions of BSF larvae were started with artisanal fish farmers from surrounding villages. Also, as a visionary man, he had foreseen the numerous potential uses of BSF. Since then, about 10 years ago, he has become a fierce advocate of BSF, together with the colleagues of his research unit; thereafter IRD and Indonesian partners gave him the opportunity to perform large-scale experiments: the bioconversion project was born. As a tireless master-builder, he has built, expanded and promoted the domestication and production of BSF in Indonesia, proving relentlessly to Indonesian decision-makers, students and fish farmers the interest and reliability of domestication and production of this insect. Saurin Hem has been and still is an atypical and determined researcher, as he thinks that science has to produce reachable goals in the near future. He made us proud to write this handbook, which is largely dedicated to him.

[1] BPPKP: Badan Penelitian dan Pengembangan Kelautan dan Perikanan

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras

Chapter 1

Biology of *Hermetia illucens*

Emilie Devic[(a)] and Melta Rini Fahmi[(b)]

(a) UMR226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan No. 13, Pancoran Mas, 16432 Depok, Jawa Barat, Indonesia

Classification and Distribution

Hermetia illucens (Linnaeus, 1758) known as the Black Soldier Fly (BSF) is an insect belonging to the order of Dipterans, family of Stratiomyidae, sub-family of Hermetiinae. The table 1-1 lists characteristic anatomical features of the species (Table 1-1).

Native of tropical, subtropical and temperate regions of the American continent, BSF is nowadays present in the rest of the world, between latitude 40° south and 45° north, and has been found in many countries across Europe, Africa, Oceania (Australia and New Zealand) and Asia (Indonesia, Japan, Philippines and Sri Lanka).

Table 1-1 Description of anatomical specific features for each subdivision of classification according to Martinez (1986), Maddison and Schulz (2007)

Classification		Specific anatomical features
Kingdom	Animalia	
Phylum	Arthropoda	Articulated invertebrates and provided with jointed appendages
Subphylum.	Hexapoda	Number of legs: 6
Class	Insecta	Insects
Subclass	Pterygota	Two pairs of wings: on the second and the third thoracic segments, a mandibular joint with condyles and metamorphosis in the course of their development
Infraclasse	Neoptera	Capacity to fold up wings towards the back, on the abdomen
Order	Diptera	Two pairs of wings: a pair of functional, flight wings with a membranous structure on the mesothorax, and a pair of halteres, derived from the hindwings on the metathorax, which help to balance the insect during flight
Suborder	Brachycera	Reduction in the number of flagellomeres (subdivision of the flagellum, last segment of the antenna of arthropods) in 8 or less. Larval mandible formed by two distinct parts.

Table 1-1 Description of anatomical specific features for each subdivision of classification according to Martinez (1986), Maddison and Schulz (2007) (return)

Classification		Specific anatomical features
Kingdom	Animalia	
Family	Stratiomyidae	Specific nervation of wings with formation of a discal cell
Sub-family	Hermetiinae	Eighth antennal flagellomeres long and thick and absence of spine on the scutellum (plates at the back of the 2nd segment of the thorax)
Genus	Hermetia	Eighth antennal flagellomeres flattened laterally
Species	*Hermetia illucens*	Very long antennae due to the exceptional lengthening of the last article of the flagellum. Abdomen with, on its 2nd segment, a pair of translucent mirrors. Smoky black wings. Large very characteristic species, 12 - 17 mm.

Human migrations and trade of goods have contributed to its dispersion (James, 1935; Leclercq, 1997). This insect, considered as non-pest, does not appear in the list of disease carrying organisms or vectors for pathogens.

Some cases of myiasis caused by larvae of *H. illucens* have been reported (Adler and Brancato, 1995; Lee *et al.,* 1995; Fuentes Gonzales and Risco Oliva, 2009), but are just exceptional.

Anatomy

Hermetia illucens is a holometabolous insect: the transition from the larval stage to the adult stage happens following a passage through a nymphal stage. Transformation is complete, and larvae and adults have contrasting morphologies and life habits. Larvae are saprophagous and photophobic (Everest Canary, 2009): they live and feed on organic matter (animal or plant) in decomposition (James, 1935).

Their cephalic capsule is separate from their body; their strong mouthparts serve eating purposes but also contribute to their locomotion. The larval body comprises 11 segments covered by hairs and bristles. Its colour is beige or light brown until pupation, thereafter it turns to dark brown. Larvae can reach up to 20 mm in length and about 6 mm in width (Figure 1-1).

Figure 1-1 Eggs and larvae of Hermetia illucens in the process of their development (from 1 to 20 days). Chromatic differences until the 17th day are a photographic artefact. On the other hand, the colour shifts from beige to dark brown from day 17 onwards

Figure 1-2 Adult BSF fly

Adults measure from 13 to 20 mm in length, they possess two long antennae, a single pair of well developed wings (when at rest wings are folded up to one on the other), as hind wings are tiny (halteres, as in all dipterans), and three pairs of legs with a white/yellow tarsus (Figure 1-2). Males are smaller than females (Tomberlin *et al.,* 2002) and an anatomic difference on the last abdominal segment enables to discriminate between genders. Females possess a retractile tubular oviduct whereas males exhibit an aedeagus (male insect reproductive organ) and a pair of hooks which enable him to grasp the female genital organ during copulation (Figure 1-3).

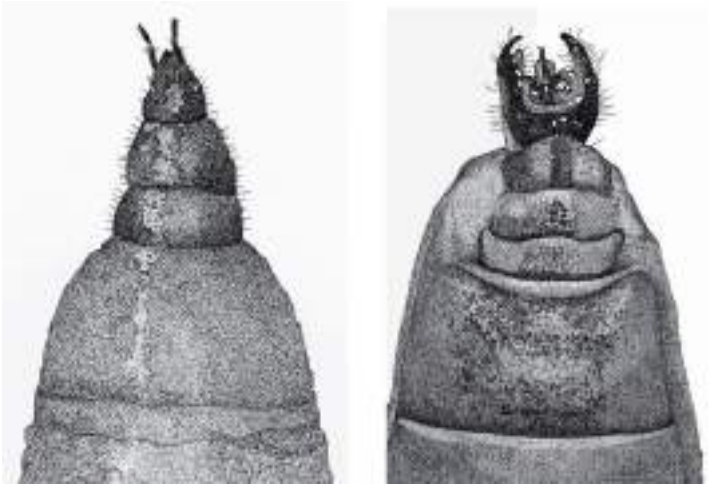


Figure 1-3 BSF Female genitalia (left) and BSF male genitalia (right)

Life Cycle

The BSF life cycle varies between populations (wild or domesticated) and environments (temperature, humidity, light intensity, quality and quantity of available food). The females lay between 320 and 1000 eggs, on a dry substrate in a humid environment (to limit the water losses of their eggs) using their ovipositor (Olivier, 2004; Kim *et al.,* 2008; Tomberlin *et al.,* 2009).

Eggs are laid into tight rows, generally in an interstice to hide them from predation and close to a potential food source. BSF females lay their eggs close to those of other individuals, and they die soon after oviposition (Tomberlin *et al.,* 2002). Eggs have an ovoid shape, and are about 1 mm long. They change from beige to yellow/beige colour during the incubation period, which lasts from a little bit more than 4 days, at 27-29°C (Booth and Sheppard, 1984) to about 3.5 days at 30°C (Tomberlin and Sheppard, 2002).

Figure 1-4 Life cycle of BSF

As soon as larvae have hatched (0.66 mm long), they use surrounding organic matter as a food source. The duration of the larval stage lasts from 4 weeks to 5 months, depending on food availability (Furman *et al.,* 1959). Temperature is also a key parameter for larval development and survival rates, the optimum temperature being in the 20-30°C range (Tomberlin *et al.,* 2009; Everest Canary, 2009). The growing larvae perform successive moults that separate five larval stages. They reach successively the pupa then the imago stage following the imaginal moult.

Morphologically, the first four larval stages are difficult to differentiate (except for body size) but the prepupal stage (5th larval stage) is

characterized by a marked colour change from beige to dark brown, a migration out of substrate (made easier by a modification of mouth organs in hooks), as old larvae cease feeding prior to pupation (Schremmer, 1986; Diener *et al.,* 2011). The last stage refers to pupae, which are 12 to 25 mm long (May, 1961 in Tomberlin, 2001; Hem *et al.,* 2008; Rachmawati, 2010). They lie motionless; their cuticle is rigidified and rich in salts of calcium forming a dark envelope.

Generally, the metamorphosis is completed within 2 weeks (Furman *et al.,* 1959) and males often emerge earlier than females (Tomberlin *et al.,* 2002; Kim *et al.,* 2008). After emergence, young adults take off after few minutes, after they have unfolded their wings. BSF is an eurygamous insect, as a consequence, it needs broad areas for its nuptial flight). Mating takes place about 2 days after the emergence of the imago (Tomberlin, 2001; Tomberlin *et al.,* 2009; Tomberlin and Sheppard, 2002), and another 2 days are needed before egg laying. An imago lives only 5 to 14 days, its life expectancy being unquestionably dependent on body size (and associated energy reserves) and on access to water (Tomberlin *et al.,* 2002; Olivier, 2004; Tomberlin *et al.,* 2009).

Physiology and Feeding

BSF adults do not need feeding as they largely live on the energy reserves built during the larval development (Newton *et al.,* 2005a, b), but these supplies can be supplemented by nectar. However, the environmental factor that intimately governs their life expectancy is the access to water: adults having access to water may live up to 14 days, whereas those deprived of water hardly survive more than 8 days (Tomberlin *et al.,* 2002).

BSF larvae are voracious until 21-24 days after hatching. The substrate in which they live is saturated with water, so dehydration is reduced and the access to food is straightforward. However, if the substrate becomes excessively humid, larvae tend to go away. Their feeding activity reduces the volume of organic matter by 40 to 80% (Diener, 2010) and strongly

impacts the substrate's content in nitrogen and phosphorus. BSF larvae are not cannibalistic.

Digestion – Enzymatic and Bacterial System

BSF larvae are polyphagous. This polyphagia, which enables them to make the most of many food sources, largely owes to their powerful mouthparts and efficient enzymatic activity of their digestive system (gut and salivary glands). It is especially in the intestine that enzymes such as amylases, proteases and lipases are most active. Other enzymes such as leucine arylamidase, α-galactosidase, β-galactosidase and α-mannosidase, not present or poorly active in domestic fly *Musca domestica,* are strongly effective in BSF larvae (Kim *et al.,* 2011).

Besides, BSF larvae host an intestinal flora that takes an active part in the digestion of food. The identification by pyro-sequencing of bacterial communities of BSF larvae's intestine has provided evidence for a specific intestinal flora, which largely differs from those documented in other insects. This flora comprises four phyla (Bacteroidetes, Firmicutes, Proteobacteria and Gammaproteobacteria) in variable proportions, depending on food sources. Among these bacterial communities, some strains or species have a substantial enzymatic activity (protease, amylase, cellulase, lipase), such as *Bacillus amyloliquefaciens, B. stratosphericus* and *Proteus mirabilis* (Jeon *et al.,* 2011).

Behaviour

Usually, adults BSF are found near places where manure, decaying food or dead animals are abundant. Smells from decaying organic matter attract females that are ready to lay eggs, whereas males prefer sunny places where vegetation prevails (Figure 1-5). Also, males are strongly territorial in that they defend their area against other males until mature females in search of a mate pass nearby (Tomberlin and Sheppard, 2001).

Figure 1-5 Adult of BSF on Sphagneticola sp. Notice highest BSF density on sunny leaves.

The life of a BSF imago being very short, mating takes place no more than 2-3 days after emergence. Mating is exclusively diurnal and comprises several steps: first the male grabs a female flying over its resting area with its legs and hooks of the aedeagus, then male and female will perform a courtship flight which can lead them up to 1.5 m above ground level where copulation starts (Tomberlin and Sheppard, 2001). Thereafter, they return to ground level, for 20 to 30 minutes, and continue forming a pair, but here male and female look in opposite directions (Figure 1-6).

Reproduction steps are governed by environmental conditions and by time of the day (Tomberlin and Sheppard, 2002); in particular, illumination and space availability are crucial during mating (Tomberlin and Sheppard, 2002; Kim *et al.,* 2008; Zhang *et al.,* 2010). The oviposition depends

mainly on the temperature (optimum from 27.5°C to 37.5°C; Booth and Sheppard, 1984) and on humidity (30 to 90%; Sheppard *et al.,* 2002).

Figure 1-6 Copulation

Current knowledge of the biology of BSF provides a framework for starting their rearing in captivity, but it largely remains fragmentary to many respects, in particular for quantitative issues that are central to production. The experimentation of the pilot production has resulted in significant progress in the process of domestication but it also raised several biological and technical issues for which the answers will enable the optimization of BSF rearing.

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras

Chapter 2

Presentation of pilot production

Domenico Caruso[(a)], I Wayan Subamia[(b)]

(a) UMR 226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan No. 13, Pancoran Mas, 16432 Depok, Jawa Barat, Indonesia.

Schematically, production is structured into two main sections: "production of adults and eggs'' on the one hand, and "grow-out of larvae" on the other hand. These two sections are interdependent, but they take place into two physically distinct structures, namely the insectarium and the larvarium (Figure 2-1).

Figure 2-1 Rearing facilities for BSF in Depok (Indonesia). Larvarium (left); Insectarium (right)

It is indeed indispensable that parents be separated from the larvarium, otherwise adult females would be strongly attracted by the production substrates and would lay their eggs anarchically, thereby complicating strongly egg collection and overall production management.

Thus, the larvarium is devoted to the grow-out of larvae and to the production of pupae, a part of which being transferred into a puparium inside the insectarium to ensure the periodic renewal of broodstock. The insectarium is dedicated to reproduction and collection of eggs, which are transferred to the larvarium for a new cycle of production of larvae and production of pupae. It is important to underline that the larvarium and the insectarium in Depok were built from existing structures, thereby imposing structural constraints upon their design. If they had been built from scratch, their design would have been different.

Other structures used in the process of BSF production, such as the place to store PKM, are not described here but are taken into account in the calculation of investments (chapter 9). The different stages of the production process that have been performed in the production pilot at Depok are detailed in Figure 2-2.

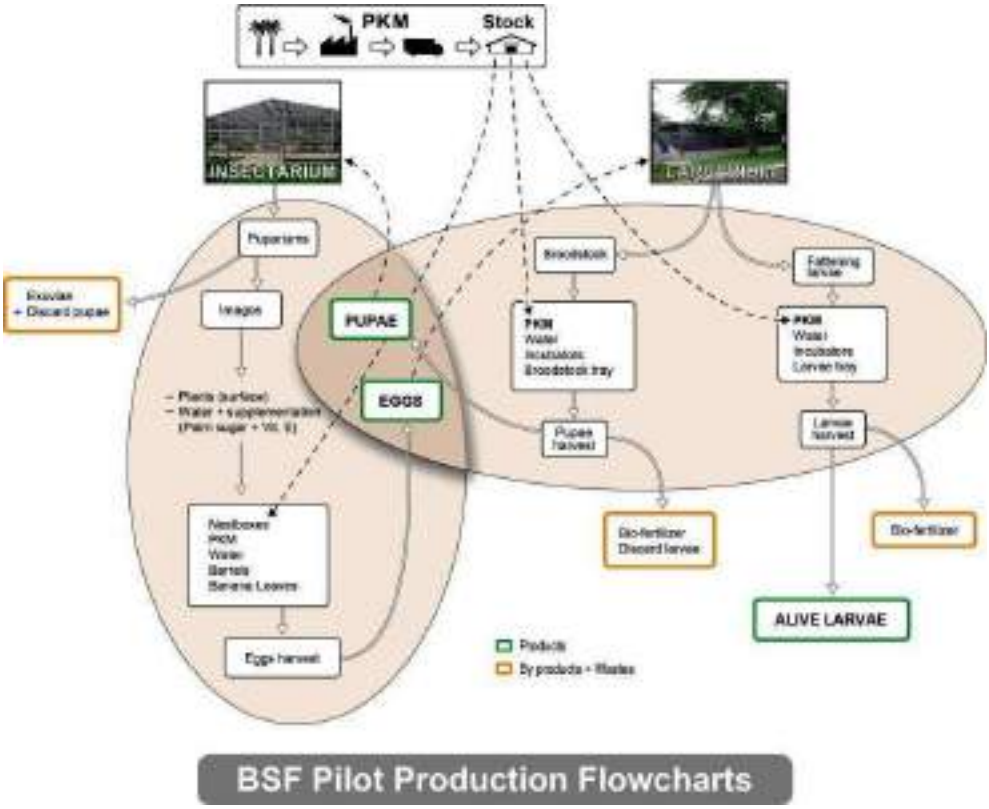


Figure 2-2 Diagram of the production system

Larvarium

The larvarium building occupies a surface area of 165 (15.6 x 10.6) m^2 with an average height of 2.4 m. Pillars in reinforced concrete (30 x 30 x 100 cm) poured into the ground (Figure 2-3a) hold metal profiles with a rectangular (10 x 5 cm) section. The lower ends of these metal profiles are fitted and welded into another slightly larger piece of metal profile, and further affixed to a metal plate that is bolted on the pillar (Figure 2-3b). Load-bearing metal bars are bolted on the top of pillars. They form the roof framework (Figure 2-3c).

The roof has a single slope: it is made of corrugated iron covering a 5-mm thick insulation material that is screwed onto the roof framework to reduce temperature variations.

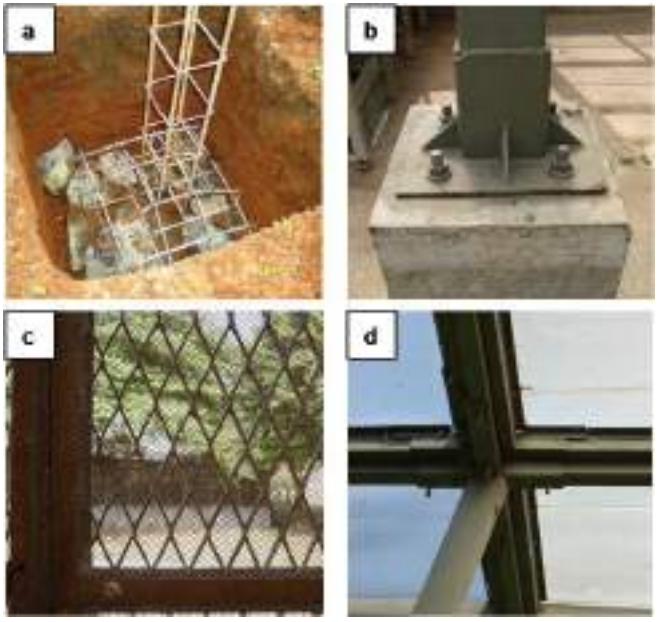


Figure 2-3 **a) Detail of the foundations of larvarium and of insectarium; b) base of metal section of a pillar c) upper section of the pillar supporting the metal section that makes the framework of the roof. To note to the left the wire mesh of the roof and to the right the polycarbonate structure (Insectarium) d) double wire netting**

The walls are made of concrete blocks covered by cement in their lower part (1 m above the ground), whereas the upper part is made of two types of wire mesh set next to each other. The first one, a large wire mesh (mesh size: 4.5 x 2.2 cm) is welded to corner irons that are bolted on the horizontal metal bars set between the concrete pillars, and serves to strengthen the structure. The second one is a fine wire mesh (mesh size: 5 x 2 mm: Figure 2-3d) that prevents the entrance of harmful insects.

This method of building guarantees a good ventilation of the larvarium, which is just indispensable as substrate fermentation produces heat. The floor of the larvarium is made of rough polished cement on which are built the “digesters”.

Here, digesters are made of blocks covered by cement (they were previously used as fish rearing tanks). Every rearing tank has a circular shape, with a diameter of 3.2 m (surface area of 8 m^2) and is 0.5 m in height. It includes a water inlet equipped with a flowmeter to measure the water volume used during the rearing cycle, which runs until larvae become pupae (Cf. Chapter 5). An additional rectangular tank has been built around every circular digester, so the overall surface of pupa production has been increased from 48 to 128 m^2 (about 75% of the ground surface) (Figure 2-4). The remaining part of the area is made of corridors and working zones.

Figure 2-4 Inside view of larvarium

The other part of the larvarium is dedicated to the mass production of larvae to be used as live food for fish. It includes 4 rectangular (4 x 1 m) tanks in polyester resin reinforced by glass fibre and covered with gelcoat.

In contrast to the digesters, which were built from existing structures, these tanks were designed for BSF mass production.

They rest on metal supports that are protected from oxidation due to humid soil by cement blocks. In order to save space, they were set across the rectangular tanks used as digesters and the corridors.

Insectarium

The insectarium is a large building with a significant wind surface area (Figure 2-1b). Its design took into account anti-seismic standards, as well as the capacity to withstand strong winds, after a lighter construction prototype (168 m^2, 6 m in height; roof frame in bamboo covered by fine mesh net of 0.5 mm) had been wrecked by gusty winds (Figure 2-5). Therefore, a more solid construction combining reinforced concrete and a metallic structure was built to face harsh tropical climatic conditions and risks of earthquake.

The insectarium occupies an area of 1,038 (44 x 23.6) m^2, with an average height of 3.2 m, thereby giving a volume of 3,364 m^3. It was built on former fishponds (22 ponds from 9 to 53 m^2) so it rests on soil and stone backfill, partly cemented, on which a floating reinforced concrete slab was poured. Fishponds were not filled in, but their walls were strengthened and cemented. On the other hand, the bottom remained intact so to plant *Sphagneticola sp.* (family Asteraceae), as adult *Hermetia illucens* are fond of their flowers. The insectarium comprises a wide central corridor and narrow peripheral and transversal corridors giving access to all sides of fishponds. Two cemented areas, one at the entrance of the building and the other one at the opposite end, are dedicated to various daily work operations.

Figure 2-5 Above: original prototype of the insectarium in bamboo and fine wire mesh. Below: the same after violent winds

The general building principles are similar to those of the larvarium, but with a more robust design. The supporting structure comprises 96 pillars at 3.6-m intervals in length and 3-m intervals in width (Figure 2-6).

Made up of two parts, a concrete base and a metal upper structure, pillars are similar to those of the larvarium, although they are much larger in every dimension. Longitudinal and transverse girders, bolted on the end of pillars by a metal cross-shaped support, are arranged along the longitudinal and transversal axes of the insectarium to form the roof frame. Longitudinal metallic joists were welded to the transversal girders to strengthen the structure.

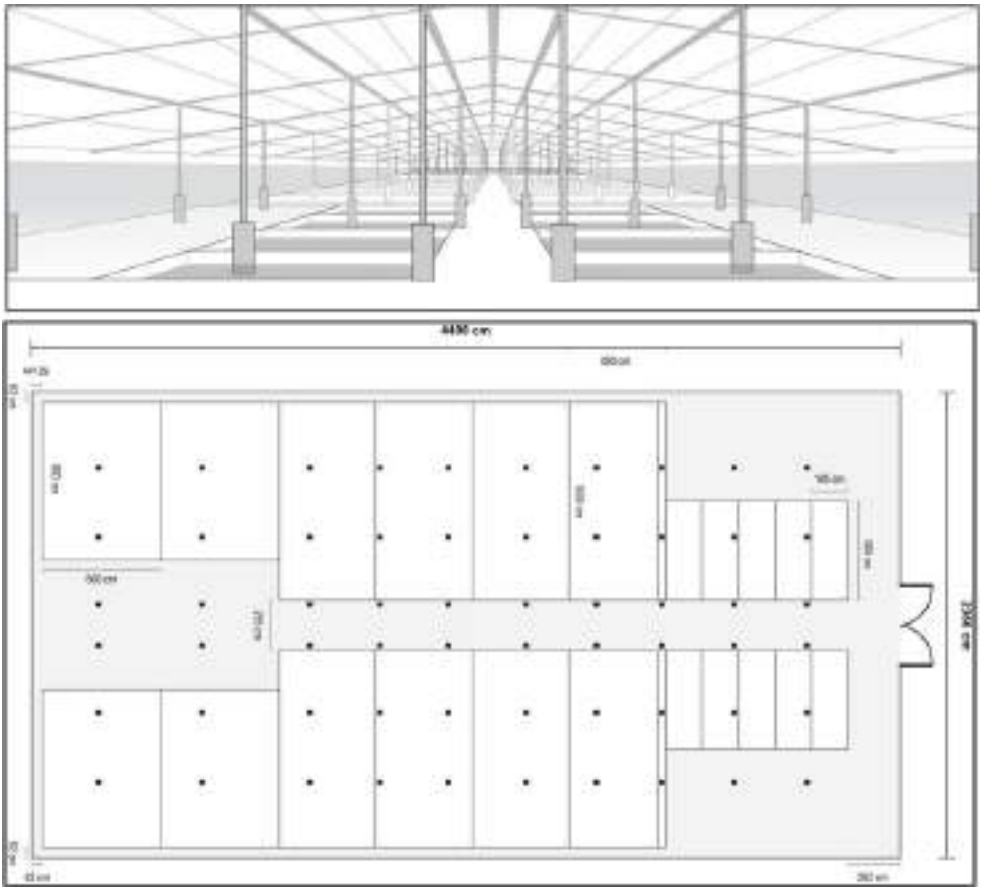


Figure 2-6 Schematic view of inside (above) and overall building plan (below) of the insectarium

The roof is asymmetric, with an average 10° slope. In addition to the main roof frame, the roof structure is further strengthened by large metallic frames that are welded and divided into 12 sub-frames. Every metallic frame is inserted between two transversal girders, and further welded and cramped by bolts to provide maximal resistance to vibration

from wind or earthquake. Transversal bolts make the adjacent frames mutually dependent. The roof cover is partly made of translucent alveolar polycarbonate sheets and partly of fine wire netting that are screwed on the frames from outside.

We also used iron rods inserted into polycarbonate sheets and screws to prevent the structure from being blown by the wind. The polycarbonate sheets are set above the corridor and on the 2/3 of the roof's lower part. On the other hand, the part of the roof over the vegetation zones is made of fine wire netting only. Finally, the walls of the insectarium are similar to those of the larvarium.

Inside the insectarium, other elements are necessary for BSF rearing such as the puparіums described below and the nest boxes, a detailed description of which will be given in Chapter 4.

Puparіums

These structures are necessary to ensure the smooth running of the insectarium and to protect pupae during metamorphosis. Twenty-four similar puparіums are aligned in two rows on each side of the insectarium and near vegetation zones. Made of wooden structures, they are 2.2 m length, located 90 cm above the ground and resting on four metallic feet. Their section is built on a trapeze-shaped plan (0.4 x 0.3 x 0.9 m). Each puparium is closed by an articulated lid that is covered with polystyrene in order to insulate the inside from ambient heat. Two longitudinal openings protected by wire mesh (mesh of 3.5 x 1.5 cm) allow emergent insects to leave the puparium while preventing the entrance of predators (lizards and rats, essentially).

Each puparium (Figure 2-7) comprises two removable trays that make the total surface twice as large (thus about 3.96 m^2) in order to produce about 15 kg of pupae in the puparium, that is to say 3.8 kg m^{-2}. A larger amount of pupae per unit of surface can compromise the emergence of adult BSF.

Figure 2-7 Pupariums inside the insectarium with removable trays for pupae disposal

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras
 ISBN: 978-979-493-610-8

Chapter 3

Substrates for rearing

Pascale Talamond[(a)] and Sukarman[(b)]

(a) UMR226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan No. 13, Pancoran Mas, 16432 Depok, Jawa Barat, Indonesia.

Substrates for Rearing

This chapter introduces growth substrates for BSF larvae rearing. The substrate principally used in Depok's production pilot is Palm Kernel Meal (PKM), obtained by extraction of seeds of the oil palm. This by-product of palm kernel oil industry is widely spread in Indonesia that is the world's largest producer of palm oil.

In view of its abundance and of the results of preliminary experiments, PKM has been selected as a valuable substrate for bioconversion. However, this substrate must be transformed before becoming a nutritional source for BSF. The first step of this transformation is the degradation of PKM, which is spontaneous process.

Oil Palm (*Elaeis guineensis* Jacq.)

Oil palm is the world's largest source of vegetal fat, ahead of soybean. It is one of the main oleaginous plants cultivated in the tropics, together with the coconut palm. Oil palm is cultivated for its two oils, which are used as fresh food or for industrial purposes. Its culture has been continuously expanding over the past 30 years. In Indonesia, the surface dedicated to palm oil plantations has increased from 3 to 7 million hectares in between 1998 and 2007. Indonesia then became the world's largest producer of palm oil, ahead of Malaysia. To meet the increasing demand of palm oil (40 million tons expected in 2020, against 25.4 million nowadays; USDA, 2011), producers further expect to expand this surface to about 20 million hectares by year 2020. This is about one third of the surface of France (PT Bakrie - Sumatra, ICE-PO, 2011). From the fresh fruit bunch one can produce: 1) 18 to 28% of red palm oil (CPO, Crude Palm Oil) that is extracted from fruit pulp, 2) 1.9 to 6% of palm kernel oil (CPK, Crude Palm Kernel) that is extracted from fruit nut, and 3) 72 to 76% of oil factory by-products including raffles, fibres, and both liquid and solid effluents. Palm Kernel Meal (PKM) amounts to 2.1% of the weight of fresh fruit bunches (Figure 3-2). Recent data indicate an annual PKM production in Indonesia of about 3.5 million tons, of which 85% are dedicated to export (USDA, 2011).

Composition of Palm Kernel Meal or PKM

Palm kernel meal (PKM) is a by-product of palm oil industry that is obtained following the extraction of oil from the kernel of oil palm fruit (Figure 3-1). It is also known as PKC, Palm Kernel Cake. PKM is a dry and gritty solid residue that is highly fibrous but still contains some oil. Extraction of palm oil is performed on the production site within 48 hours following harvest, after steam sterilization, desteming of fruit bunches, crushing and finally decantation. The nut is crushed, screened and boiled, then pressed to extract palm oil.

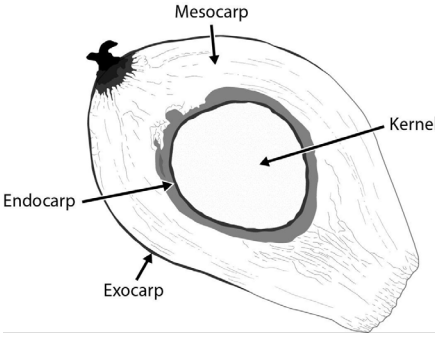


Figure 3-1 Section of the fruit of oil palm (Elaeis guineensis)

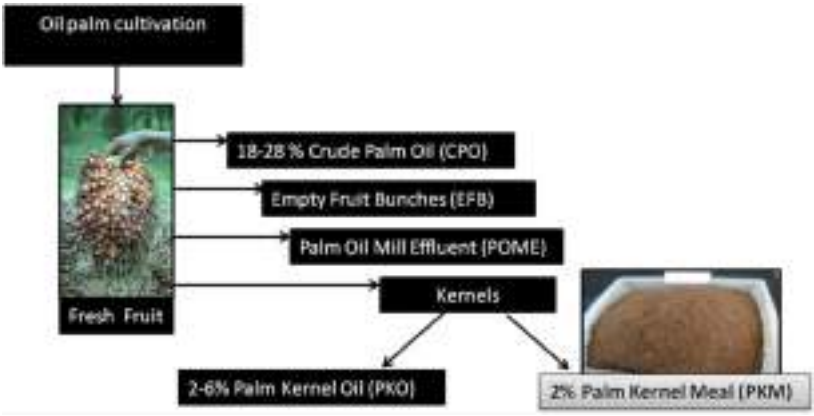


Figure 3-2 Production of palm oil and its by-products

The PKM used in Depok production pilot was provided by PT Perkebunan Nusantara, located at Bandar Lampung (southern Sumatra, Indonesia). PKM is produced by dry extraction process and has a dry matter (DM) content of about 92%. Its proximal composition, as determined using the AOAC international procedure (1998), is given in Table 3-1.

Table 3-1 Proximate composition of palm kernel meal PKM[1] n=9 between 2008 and 2011, Talamond et al., 2011[1]; Alimon, 2004[2]; Ng, 2004[3]; Vilariño, 1996[4]. Neutral Detergent Fibre (NDF) using Van Soest[5] method

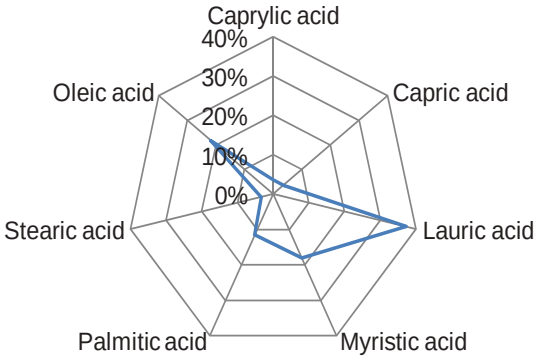
Nutrient composition	PKM[1] (Indonesia)	PKM[2] (Malaysia)	PKM[3] (Malaysia)	PKM[4] (Venezuela)
Dry Matter (DM, %)	92.5 ± 2.1	88.0 – 94.5	88.6	92.5
Ether Extract (Lipids) (% DM)	9.8 ± 3.0	5.0 – 8.0	6.8	14.5
Ash (% DM)	4.6 ± 0.2	3.0 – 12.0	6.6	4.5
Crude Protein (% DM)	16.7 ± 1.2	14.5 – 19.6	16.9	13.5
Crude Fibre (% DM)	23.6 ± 5.5	13.0 – 20.0	15.1	-
NDF[5] (% DM)	68.8	66.8 – 78.9	-	60.6

Crude protein, ash or mineral matter contents vary little between PKM from different origins. By contrast, lipids and fibre contents are more variable between origins, but these differences can also reflect the use of different methods for oil extraction.

Most fatty acids in PKM are saturated, except for oleic acid (C18:1) which is the only unsaturated fatty acid (Graph 3-1). The amino acid profile of PKM is rich in both arginine and glutamic acid, but poor in lysine and sulphur amino acids (methionine and cysteine) (Table 3-2). The availability of amino acids in PKM is about 65 %, thus lower than in most oleaginous cakes (Alimon, 2004; Mustafa *et al.,* 2004).

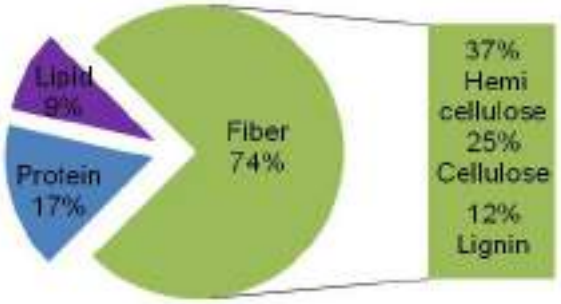
Table 3-2 Amino acid profile of palm kernel meal (PKM)

Amino acid content (g/16 g N)	References			
	Alimon, (2004)	Hem *et al.*, (2007)	Sundu *et al.*, (2008)	Moreau, (2010)
Alanine	3.8			
Arginine	11.6	13.9	19.2	26.1
Aspartic acid	3.6			
Cysteine	1.1			
Glycine	4.2		6.5	
Glutamic acid	16.8			
Histidine	1.9	2.5	3.2	3.5
Isoleucine	3.2		5.5	7.4
Leucine	6.1	6.4	10.4	13.3
Lysine	2.7	3.7	4.0	7.10
Methionine	1.8	2.7	1.7	
Phenylalanine	4.0	3.6	6.4	
Proline	3.3			
Serine	4.1		7.8	6.6
Threonine	2.8	3.5	5.3	2.0
Tyrosine	2.6		2.4	
Valine	5.1	5.7	7.7	11.2



Graph 3-1 Fatty acid composition of PKM

Palm kernel meal is a highly fibrous substrate. The PKM used for BSF rearing in Depok has a fibre content of 74%, of which 62% are polysaccharides (cellulose and hemicellulose) and 12% lignin (Graph 3-2). The main polymer of hemicellulose is mannan that makes 78% of the total structure of PKM polysaccharides (Düsterhöft *et al.,* 1992, Cerveró *et al.,* 2010). The complete hydrolysis of this polysaccharid releases mainly mannose. These molecules constitute complex and highly resistant associations that make PKM poorly digestible for monogastric animals such as poultry and fish. This high fibre content limits the use of PKM to a maximum of 15% for chicken feeding (Berepubo *et al.,* 1995).



Graph 3-2 Hemicellulose, cellulose, lignin and crude fibre contents of PKM (% DM).

Some researchers have tried to improve the nutritional value of PKM using an enzymatic pre-treatment. Others have used microorganisms such as fungi for the formulation of food dedicated to both animals and the production of biofuel (Ng *et al.,* 2002; Muangkeow and Chinajariyawong, 2009; Jørgensen *et al.,* 2010; Wong *et al.,* 2011). Whatever the system used, either enzymatic or microbial, transformation allows the partial digestion of fibres and improves the bioavailability of nutrients in the substrate.

PKM Degradation for Rearing Larvae of *Hermetia Illucens*

Larvae of the black soldier fly *Hermetia illucens* feed naturally on decaying animal or vegetal substrates. Two degradation processes have been used in the production pilot at Depok, i.e. aerobic *vs.* anaerobic. The former process is used to prepare the growth substrate of fattening larvae and production of BSF pupae for broodstock renewal. The latter degradation process is used both as a growth substrate for BSF larvae aged 0-6 dah and as an attractant for egg laying.

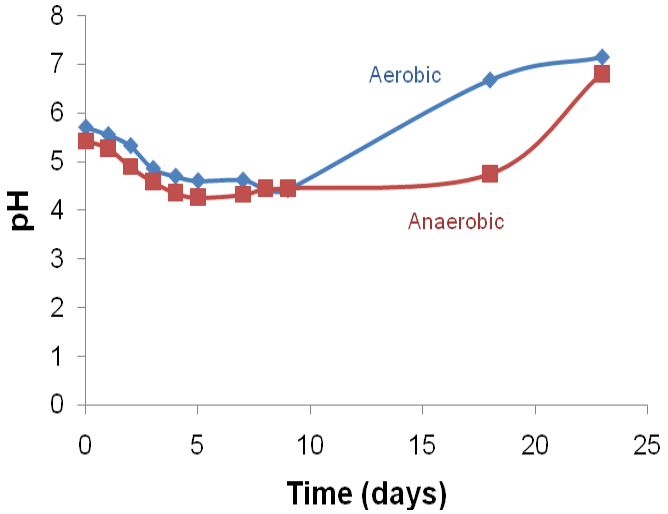
Aerobic Degradation

PKM is mixed with water in a 1:2 ratio (1 kg PKM - WM - for 2 L of water), then it is poured into the digester in 5-6 cm thick layers. At the end of this process, the digester is covered with a mosquito net that prevents the intrusion of undesirable insects. The initial humidity of PKM is about 60%. Its pH is between 5 and 6. As a result of ambient humidity and temperature inside the larvarium, fungi colonize the substrate surface in a few days (Figure 3-3). Three main types of moulds have been identified in the growth substrate: *Aspergillus, Penicillum* and *Mucor* sp. (Pangestu, 2009). Fungi of the *Aspergillus* genus can produce aflatoxins, but these compounds were never detected either in the substrate or in BSF larvae during rearing (Test Rida Quick Aflatoxin, r-biopharm®). Additional water supply on a regular basis can be necessary to maintain the humidity rate and prevent the substrate from drying.

PKM degradation provides BSF larvae with an adequate supply (*cf.* chapter 8). First, larvae feed on directly available nutrients of PKM, among which proteins and lipids. Then, they will find additional food sources after other nutrients, mainly digestible carbohydrates, have been produced by the enzymatic activity of micro-organisms on PKM polysaccharides. Other enzymes also have an action on proteins and lipids. The degradation

process also involves the acidification of the substrate, which can be measured by pH variations. After 3 days, the substrate pH decreases by one unit with respect to its initial value (pH of 5.7), then it remains constant from the 5th day onwards (Graph 3-3).

Figure 3-3 **Degradation of Palm Kernel Meal, (PKM) for production: a) aerobic condition, b) anaerobic condition**



Graph 3-3 **Variation of growth substrate acidity (pH) in aerobic and anaerobic conditions during BSF larvae feeding**

When other substrates such as copra residues are used, pH can be as low as 3-3.5 within five days after the beginning of degradation. Acidification can have several origins; it may result from the production of organic acids, carbohydrates, lipids or other substances. During the aerobic degradation of PKM, the production and dissolution in water of CO_2 further contributes to the acidification of the substrate. After 10 days of BSF larvae feeding, the substrate's pH progressively increases and eventually becomes neutral, probably as a result of the occurrence of nitrogen compounds produced by BSF larvae.

At the end of the production process, the characteristics of PKM residues differed substantially, depending on whether PKM was used for the production of BSF pupae or fattening larvae.

As regards the production of pupae, PKM residues are in the form of dry pellets (80-84% DM) and are constituted by some of indigestible substances and faeces of BSF larvae. By contrast, in the case of BSF larvae, PKM residues have a much higher water content (44% moisture content) and are not fully used. Their chemical compositions are shown in Table 3-3.

Table 3-3 Chemical composition of substrate residues from PKM following the production of BSF pupae or larvae; [1]Neutral Detergent Fibre (NDF), using Van Soest method

Nutrient composition	Pupae production residues	Larvae production residues
Dry matter (%)	82.7	43.9
N total (% DM)	24.9	22.0
Ether Extract (Lipids) (% DM)	0.7	3.0
Ash (% DM)	13.6	6.4
Crude Fibre (% DM)	38.1	34.3
NDF[1] (% DM)	70.1	-

Anaerobic Degradation

In contrast to the aforementioned situation, the anaerobic degradation of PKM mainly relies on the action of the microbial flora. Its action on the availability of PKM nutrients has been evaluated recently (*cf.* "Methods for Characterizing Fermentation"). The method used for the degradation of PKM in anaerobic conditions stands as follows: a 1:2 ratio of PKM-water mixture (55 kg WM for 110 L of water) is stored in a container (height: 81 cm; diameter: 48 cm; capacity: 160 L) entirely filled and closed by a hermetic lid (Figure 3-4).

Methods for Characterizing Fermentation

Isolation and counting of microflora was performed every 12 hours during one week on both specific and non-specific environments: TSA (Trypticase Soy Agar), MRS (de Man, Rogasa and Sharpe), RCA (Reinforced Clostridial Agar) and SGA (Sabouraud Glucose Agar). The temperature, pH and titratable acidity of the substrate were also measured (Ayuningtyas, 2012). During fermentation, organic acids have been measured using gas chromatography. Hexoses (monosaccharides with 6 carbon atoms) have been analysed using a diagnostic technology (four-enzyme coupled assay, Megazyme kit). Fibres were measured with Van Soest's method.

After one week of fermentation, the mixture can be used as a substrate for egg laying or for rearing larvae until the age of 6 dah. The mixture can be preserved and remains equally suitable for these purposes for about one month. The anaerobic degradation of PKM is a complex process that involves a large diversity of micro-organisms. Bacteria contribute to 98.6% of the total microflora, whereas yeasts and fungi only amount to 1.4%. During the first 2-3 days of cultivation, many species of micro-organisms are growing at 18 $\log_{10}$ cells CFU/g, defined by total anaerobic and aerobic bacteria (Table 3-4).

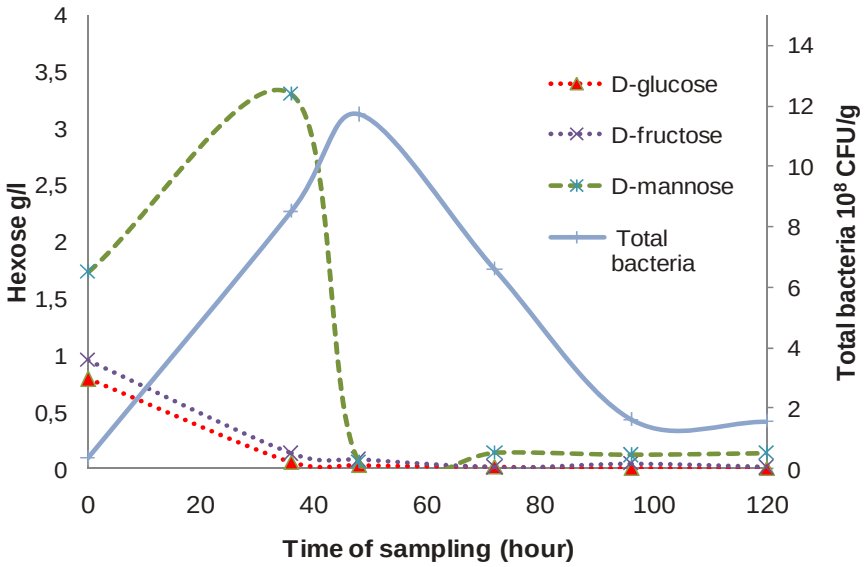
Table 3-4 **Evolution of microflora during the anaerobic degradation of PKM. TSA (Trypticase Soy Agar), MRS (de Man, Rogasa and Sharpe), RCA (Reinforced Clostridial Agar) and SGA (Sabouraud Glucose Agar); Ayuningtyas, 2012**

Time of sampling (hour)	Aerobic bacteria (log_{10} cells per g)		Anaerobic bacteria		Total bacteria (log_{10} cells per g)	Total fungi
	TSA[1)]	MRS[2)]	RCA[3)]	TSA an[4)]		
0	7.5	-	-	-	7.5	-
12	9.1	-	3.0	7.3	16.4	-
24	9.6	7.6	7.7	8.3	17.9	-
36	8.5	8.6	7.8	8.7	17.2	-
48	8.8	8.5	7.4	8.7	17.5	-
60	8.7	8.3	7.7	8.5	17.2	-
72	8.4	7.6	8.7	8.6	17.0	-
84	8.5	7.1	8.0	8.2	16.7	-
96	7.9	6.7	8.0	7.9	15.8	5.5
108	7.6	6.8	7.9	7.9	15.5	6.4
120	7.8	6.7	8.0	8.0	15.8	6.3
132	7.9	5.7	8.0	8.1	16.1	6.0
144	9.6	6.6	7.7	7.9	15.5	6.1
156	8.1	5.9	7.5	8.0	16.0	6.2
168	7.9	5.9	8.0	8.0	15.9	6.4

During anaerobic degradation, 266 bacterial strains were isolated, of which 179 were gram (+) and 87 gram (-). Lactic bacteria are enumerated on MRS agar medium and detected after 24 hours. Their concentration increases during the next 12 hours and become stable thereafter. At the end of degradation process; they prevail over any other organisms. Lactic bacteria strains (67) were isolated upon degradation. These bacteria principally belong to the genus *Lactobacillus spp.* After 24 hours of fermentation, bacterial activity releases organic acids (mainly lactic and

acetic, succinic and propionic acids) that cause the pH to fall down to 4. After 6 days, lactic acid contributes to about 90% of organic acids.

During fermentation, lignin concentration decreases by 51%, whereas hemicellulose and cellulose concentration decreases by 11%. Fungi are poorly present in the substrate, but they could be involved in lignin degradation to a greater extent than in the degradation of cellulose and hemicellulose. Hydrolysable polymers of polysaccharides are released in the environment due to enzymatic action on PKM fibres. The hydrolysis of hemicellulose releases manno-oligosaccharides, then mannose itself. Similarly, gluco-oligosaccharides and glucose are produced from the hydrolysis of cellulose. Some fructose has also been detected in the fermented substrate (Graph 3-4).



Graph 3-4 **Growth of total bacteria as a function of hexose release (mannose, glucose, fructose)**

Therefore the anaerobic degradation of PKM is a lacto-acetic fermentative process that transforms complex organic substances into simpler elements. The substrate is acidic because of the release of organic acids, which prevents the development of an alteration flora and of pathogens in the substrate. Degradation improves the properties of PKM through the pre-digestion of polysaccharides into assimilable carbohydrates and dissociation of amino acids. Moreover, lactic bacteria produce substances with an antimicrobial activity, which contribute to substrate stability and facilitate its conservation, as well as other compounds, e.g. from the vitamin B group.

For logistical reasons, the anaerobic fermentation of PKM has not been used in Depok for the production of larvae or pupae of BSF. However, experimental results on the entire production cycle suggested a higher efficiency of PKM degradation under anaerobic conditions for BSF larval rearing. Indeed, the production of BSF pupae on anaerobically degraded PKM was about 20% higher than when PKM was degraded in aerobic conditions. This difference is probably due to a higher survival rate of BSF larvae, as the growth of BSF larvae did not differ between the two degradation methods.

Anaerobic PKM degradation may have positive effect also on egg production. A preliminary result also indicates a positive effect of PKM degradation on egg production. In females BSF feeded with PKM degraded anaerobically, the oviposition was about three times higher than that obtained with same substrate degraded aerobically. Thus, the anaerobic degradation of PKM could be a way to optimize the overall production of BSF.

Other Production Substrates

BSF rearing tests have been performed with other substrates such as manure, pig manure (Newton *et al.*, 2005a; Myers *et al.*, 2008), household and municipal organic wastes (Diener *et al*., 2011), coffee pulp (Lardé, 1990), chicken feed or faecal sludge (Diener *et al.*, 2009b). Newby, (1997)

studied the opportunity of using BSF larvae for domestic compost. Most of these substrates are residues that must be eliminated by humans or recycled (Table 3-5).

Table 3-5 BSF larva production other substrates, [1]Wet Matter, [2] Dry Matter Reduction rate % in DM of PKM: [3] ($R_{distrib}$ - R) / $R_{distrib}$ ($R_{distrib}$: the total weight of distributed as food ration, R: Residue of bioconversion)

Substrate	Substrate (Weight WM[1] / day)	Pre-pupa weight (WM[1], g)	Substrate reduction rate[3] (% DM[2])	Reference
Coffee pulp	66.5	0.147	29.8	Lardé, (1990)
Pig manure	37.5	-	56.0	Newton et *al.,* (2005a)
Cow manure	27-70	0.09-0.14	33 - 58	Myers et *al.,* (2008)
Chicken food	0.012-0.200	0.137	26.2 – 39.7	Diener et *al.,* (2009b)

The aforementioned authors mainly focused on the reduction of waste than on BSF production. According to Diener *et al.,* (2009b), 3 tons of daily waste would enable the production of a larval biomass of 150 kg of BSF per day (dry weight of pre-pupae). Because of different objectives, these data cannot be compared with those of the pilot production at Depok, which essentially aimed at optimizing the production of BSF in respect to the input of PKM.

The PKM used in the production pilot at Depok is an input. However, it could be wise to consider alternative substrates for several reasons (*cf*. Chapter 9). When grown on copra residues obtained following the artisanal extraction of coconut pulp milk, BSF larvae exhibit similar growth as with PKM. However, the degradation of the coconut pulp milk must be achieved in anaerobic conditions, and it is much longer than for PKM (3 weeks *vs.* 7 days). In these conditions, copra residue can be supplied all along the production cycle, starting from the first day of BSF larval feeding. If this long degradation process is no obstacle to production, copra residues can be used as a substitute to PKM. Copra residues mixed with PKM in range 20-50% can be used as growth substrates without any negative effects on

the production of BSF pupae. It could thus replace completely or partly PKM, because its availability in Indonesia is less restricted than for PKM.

It has been tried using fish waste, already acidified with 3% formic acid, stored over 14 days in a container then mixed with PKM into a growth substrate for BSF larvae. Growth or nutritional traits of BSF larvae were similar to those obtained with a PKM substrate, except for larvae having a higher content in polyunsaturated fatty acids when growing on fish waste.

Trials with residues of tofu (prepared from soybean meal) have also been tested. However, preliminary results pointed out that this substrate requires being degraded by micro-organisms before BSF larvae can make the most of it. Several other plants with a high protein content might be used as alternatives for the production of BSF larvae (*cf.* Chapter 7).

Other aspects must be considered before selecting the right growth substrates for BSF larvae, namely their availability, quality or cost and the manpower needed for their processing before they can be used as substrates for bioconversion. It is also necessary to take into consideration the geographical location of these resources to assess the economic, energy and environmental impact of transport.

These different aspects largely overlap. The study of all these aspects will eventually decide which growth substrate is most adequate for the production of BSF in a particular context.

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras

Chapter 4

Rearing of adult BSF and egg production

Emilie Devic[(a)], Nurhidayat[(b)] and Domenico Caruso[(a)]

(a) UMR226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan No. 13, Pancoran Mas, 16432 Depok, Jawa Barat, Indonesia

Production of Pupae

Collection of eggs laid by wild adults or egg production in controlled rearing conditions is a prerequisite for the efficient rearing of *Hermetia illucens*. Indeed, whatever the purpose of the production - larvae dedicated to animal feed and/or reduction of organic wastes - it is recommended to use homogeneous larvae (age, size, weight) from the first days following hatching. This will contribute to standardize the production and optimize bioconversion. The capture of wild BSF adults is possible; however it overlooks the issues of acclimation to captivity. Moreover, considering the short life of BSF adult (5-14 days), it would have little efficiency.

On the other hand, the regular collection of eggs soon after spontaneous breeding is easier and can optimize rearing, as larvae are homogeneous in age and size. To achieve this goal, it is necessary to offer, close to the insect's natural habitat, several egg-laying containers filled with an attracting substrate. It is also necessary to collect eggs or young larvae at regular intervals (eggs hatch 3 or 4 days after oviposition). In Indonesia, several types of "nest boxes" have been used. In practice, these nest boxes contain fermented PKM upon which are set dry banana tree leaves that serve as a laying substrate and offer a protection for eggs.

During preliminary trials, these nest boxes were placed in vegetated areas (banana plantation, long grass, small bushes…) to attract wild insects (Figure 4-1).

Figure 4-1 First set-up of nest boxes in open environment (Indonesia) for the collection of eggs laid by wild BSF

A corrugated plastic sheet covered the nest box to provide shelter from rain and limit evaporation, leaving also enough space for adults BSF to access the oviposition site at their own volition (Figure 4-2).

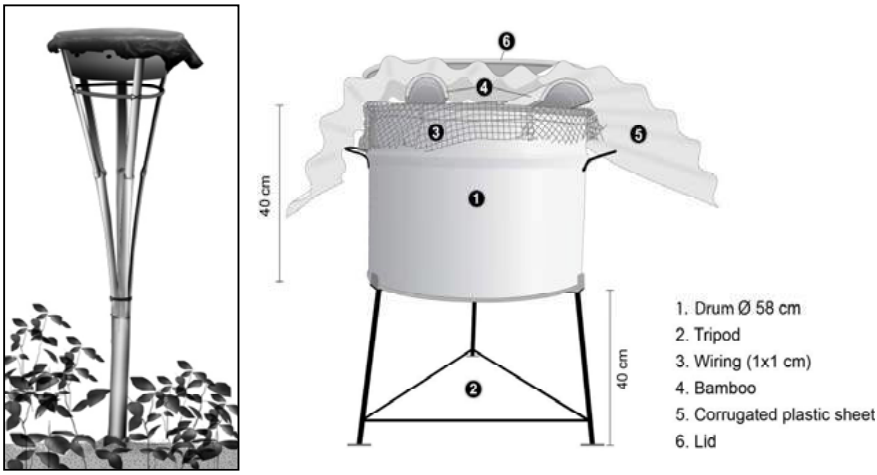


Figure 4-2 Two different types of egg-laying containers used in open environment : plastic nest box on top of a bamboo pole (left) and barrel on a metallic tripod (right)

The concentration of BSF can attract a large number of potential predators at various levels of the food chain (ants, lizards, rodents, birds, snakes…). Therefore, it is necessary to restrain the intrusion of predators by covering containers with a wire netting and by placing them well above the ground.

Oviposition Substrate

In the production pilot at Depok, fermented PKM has been used as laying substrate, but any decaying organic matter may attract BSF females for oviposition (Copello, 1926; James, 1935). During the decay process by microorganisms, various volatile attractive substances such as alcohol, aldehydes, organic acids… are released.

Concerning PKM, the main organic acids released during fermentation are the acetic, propionic and butyric acids. These compounds are known as attractants for dipterans as long as they are associated with other molecules (Hwang *et al.,* 1976; Robacker *et al.,* 1996). However, olfactometric experiments performed by Tomberlin, (2001) did not identify the specific molecules or bouquet that act as attractants for BSF.

The insect-substrate interaction is a complex phenomenon that depends on environmental factors, on substrate availability and physicochemical characteristics, and on the presence of either conspecific immature insects or competing species (Desouhant, 1997).

Furman *et al.* (1959) suggested that the presence of BSF larvae in a substrate influenced its selection by BSF females for laying its eggs; however this hypothesis has not been supported by other authors (Kemppinen, 1998; Tomberlin and Sheppard, 2002). Females of *Drosophila melanogaster* use their gustatory abilities to evaluate the quality of oviposition sites (Yang *et al.,* 2008). Whatever the underlying mechanism, it is likely that the oviposition substrate is chosen by the female according to its nutritional value for its offspring.

Experience shows that it is necessary to maintain the substrate humidity around 60-70%, as it helps releasing volatile attractive substances. It is further recommended to renew baits regularly, approximately every 10 days.

Methods of Egg Collection

In the production pilot at Depok, nest boxes are black plastic containers, with large holes on the sides covered by wire netting, and containing about one kilogram of wet PKM degraded under anaerobic conditions. Dried banana tree leaves are placed on a support wire netting above the humid substrate to prevent their humidification (Figure 4-3). A second wire netting is placed on the top of the box to prevent the entrance of predators such as rodents and some lizards, whereas adults BSF are free to come and go.

In total 58 nest boxes (offering an oviposition space of constitute several stacks made of 4 to 5 nest boxes each (height: 130-150 cm). Approximately 6 m^2) are placed above each other upon a tripod.

Figure 4-3 Typical nest box used in the insectarium at Depok. Plastic bowl with degraded PKM (anaerobic) covered by metallic wire netting and dry banana tree leaves

A small container filled with water is placed at the bottom of each foot of the tripod to prevent the intrusion of wingless insects such as ants into the net boxes. The stacks are positioned at each corner of the insectarium (numbered from 1 to 4 as shown in the diagram below) close to the vegetated areas (Figure 4-4).

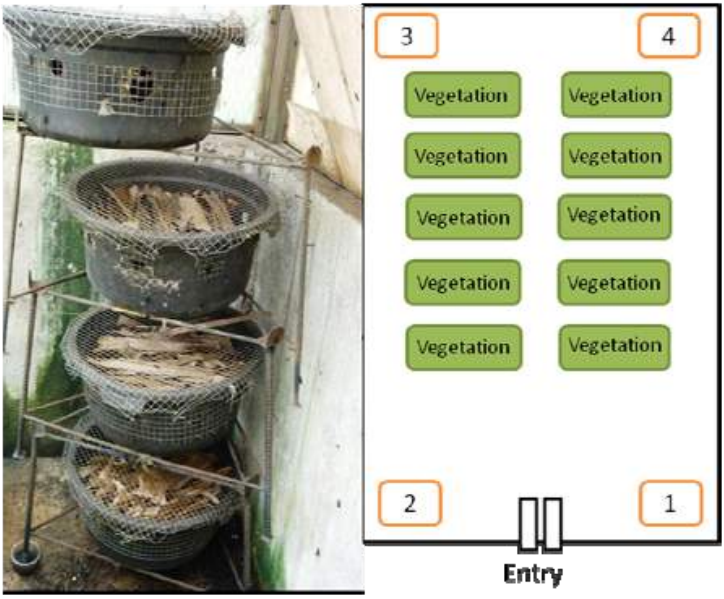


Figure 4-4 Stacked nest boxes arranged in columns inside the insectarium and diagram of the insectarium at Depok showing the locations of the nest boxes (corners 1 to 4)

In the whole insectarium, the median amount of eggs harvested in each nest box was 1.5 g (25%: 0.61 g; 75%: 2.76 g) per collection. However, productivity strongly differed between corners: the amounts of eggs harvested in corners 3 and 4 were systematically higher than in corners 1 and 2, which were close to the entrance. This can be explained by the location of nest boxes 3 and 4 closer to the vegetation growing in the insectarium and thus to BSF themselves resting on the plants.

By contrast, the position of a nest box within the column had seemingly little influence on egg productivity, except for nest boxes close to the roof (about 20 cm), which had a very low efficiency.

Eggs had to be collected before hatching, so harvests were scheduled three times a week. Egg collection was a manual operation, which required precautions and no less than three operators spending 2 to

Figure 4-5

Collection of eggs (left) and meticulous recovery of eggs using a bamboo spatula (right). The production of each nest box is individually weighed.

4 working hours each on every day of harvest. Indeed, each banana tree leaf, in every nest box, was carefully searched for eggs.

Then, eggs masses were carefully removed from the leaves using a small bamboo spatula, put into a pre-weighed container and weighed to estimate the overall productivity of the insectarium (Figure 4-5).

In Depok, two other types of nest boxes have been evaluated: 1) a plastic bag, filled with fermented PKM, offering grooves for oviposition thanks to the puckered hems that close the bag; 2) plastic or bamboo strips arranged in a fan shape placed above fermented PKM (Figure 4-6). However, these nest boxes have not been found as effective as those using the banana tree leaves that were therefore preferred, in particular for ease of egg collection.

Figure 4-6 Alternative egg collectors: a) plastic bags with hems, b) small plastic strips; c) small bamboo strips

Other Methods of Egg Collection

Among all methods used for BSF egg collection, the most frequently used is the one described by Booth and Sheppard, (1984) (Sheppard *et al*., 2002; Tomberlin and Sheppard, 2002; Newton *et al*., 2005a; Diener *et al*., 2009b; Zhang *et al.,* 2010). It is similar to the system experimented at Depok, involving plastic receptacles containing about 1 kg of humid attractive substrate and placed in the middle of the rearing structure or near the BSF natural habitat, at approximately 40 cm above the ground. The substrate used is either domestic fly larvae feed (Hogsette, 1992), poultry manure or hen feed. Bands or rolls of corrugated cardboard that offer many small cracks for the oviposition, are stuck along the upper part of the receptacles, about 3 cm above the substrate. Unlike the technology used in Depok pilot, egg masses are not systematically removed from the receptacle and the amount of eggs is evaluated by measuring the weight of the cardboards before and after oviposition. However it is necessary to make sure that these receptacles have not been humidified along the process, otherwise the amount of eggs would be overestimated.

Broodstock

Considering the short life of BSF adults, the insectarium must be regularly repopulated with new cohorts in order to avoid any disruption of egg production. Hence, several kilograms of pupae (44 ± 17 kg, depending on the success of production of the larvarium) have to be transferred every week into the puparium. It is important to avoid any disturbances during insects' emergence.

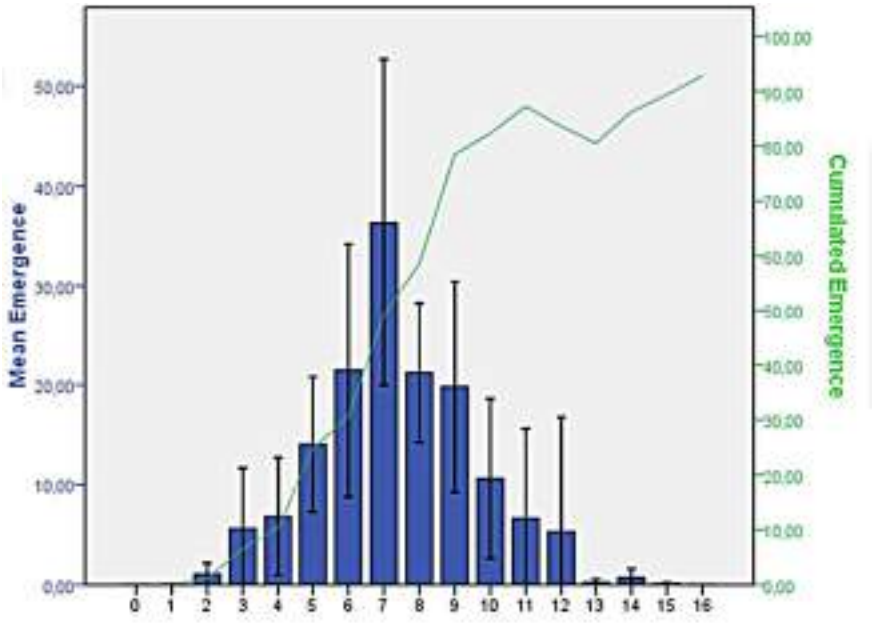
Experience shows that the pupae's layer thickness should not exceed one centimetre and the density of pupae in a puparium is about 3.8 kg/m^2.

Samplings were performed for each cohort introduced in the insectarium to estimate the amount of transferred pupae. The rate and the dynamics of emergence were also evaluated in order to estimate the quantity of adults in the insectarium.

Three samples containing about 20 g pupae, collected randomly and stored in a plastic box (120 cm^2) closed by a mosquito net, were prepared. Pupae

were initially counted and every day, emptied exuviae were evaluated and removed from each box. In total 21 cohorts were monitored and results showed that the peak of emergence (40 %) takes place on the seventh day following the transfer of pupae to the insectarium.

A cumulated emergence curved showed that 90% of the flies have emerged after 15-16 days; puparium can then be cleared (Graph 4-1). Insects that have not emerged are considered as abnormal or dead (rebuffs). The blooms of emergence are recorded mainly in the morning and on sunny days.



Graphic 4-1 BSF emergence rate and cumulated emergence rate (day 0 = introduction of pupae in puparium). Whiskers indicate IC at 95%

Breeding in Semi-controlled Environment and Maintenance of BSF adults

The environment in which BSF adults are expected to mate should be as similar as possible to natural conditions. It is recommended to grow some plants in the insectarium, as BSF appreciate resting on green leaves. At Depok, *Sphagneticola sp.* (*Asteraceae*) was selected as it does not require any particular care and it needs little water. This plant grows close to the ground and the different levels formed by the leaves offer resting and mating areas for the flies, which can enjoy the sun or protect themselves from the rain (Figure 4-8).

Figure 4-8 Adults of Hermetia illucens on the leaves and flowers of Sphagneticola sp.

Observations confirm the results of Tomberlin and Sheppard, (2001) concerning the habits of BSF adults: females are mostly found around oviposition areas, whereas males prefer resting on the vegetal covering waiting for mature females to mate (Table 4-1).

Table 4-1 Distribution of adult male and female BSF in the insectarium (n=8787)

Environment	Male (%)	Female (%)
Vegetation	59	45
Close to the nest box	41	55

The height of the cage is also a key element: Tomberlin and Sheppard, (2001) noticed that mating couples can fly up to 1.5 m above the ground in natural environments. It is thus recommended to have high ceiling in any BSF insectariums (higher than 1.5 m). Once adult BSF have emerged, limited care is required during the rest of their life.

Although not strictly indispensable to their survival and reproduction, the presence of water is beneficial to BSF as it increases their lifespan. In the pilot, water is supplied daily (about 0.08 $L.m^2$) using a pressurized water gun that allows spraying small droplets on the vegetal cover (Figure 4-9).

Figure 4-9 Water and diluted palm sugar supply sprayed with a high pressure device

Palm brown sugar is diluted in water sprayed (about 3 - 4 g. L^{-1} of water, equivalent to 10 kg sugar per month) in order to provide additional energy to the broodstock to support breeding performances. Vitamin E, known to be a fertility stimulant, is also added to the mix. However, there is no scientific evidence proving the positive effects of these supplementations in adults BSF's diets.

Tomberlin and Sheppard, (2002) showed that mating relies strongly on direct sunlight. Hence, to allow sunbeams in the insectarium and to increase the light intensity inside, some parts of the roof were replaced by metallic wire nettings. These "windows" also allow the rain to get through, but reduce the size and strength of raindrops and contribute to spray water onto the vegetation.

This has a limited negative impact on reproduction, as mating is genuinely less frequent on rainy days, due to lower light intensity and cooler temperature. Finally, it is difficult to prevent all BSF predators from entering the insectarium. Basically, several species of lizards live inside, on plants or walls (Figure 4-10). Rodents also succeed in getting inside the insectarium by cutting up wire nettings with their teeth. About once per month, the insectarium is inspected and damages are repaired; traps and baits are also used for rat extermination.

Figure 4-10 Lizard watching an adult of BSF on the leaves of Sphagneticola sp.

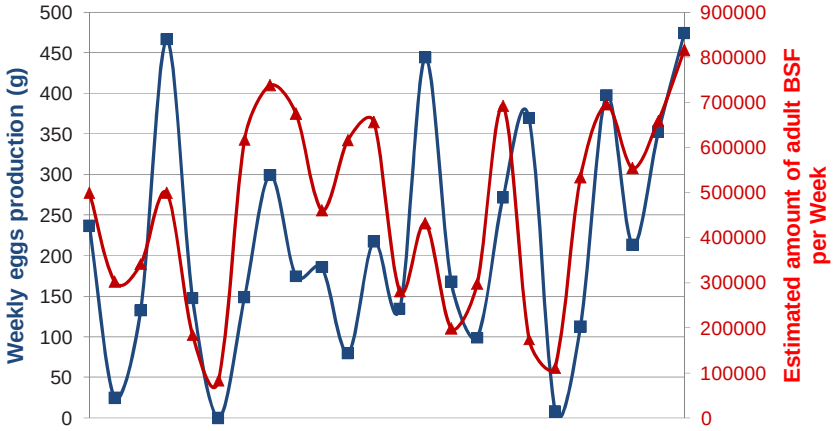
Egg Production

Every week, an average of 215 ± 142 g of eggs is collected in the insectarium. Considering the amount of pupae produced weekly from an 8 m^2 digester and introduced in the insectarium (about 464 000 BSF), one would have expected egg production to be much higher than observed here.

Likewise, if all digesters available in Depok had been used at the same time for the production of pupae, egg production would have been much higher, but this was not a priority as it would have been excessively time and resource consuming. An maximum egg production is difficult to obtain, as many factors can act negatively on the reproduction of BSF.

Sex ratio is a key parameter for the production of eggs; in Depok this ratio was clearly unbalanced in favour of males contributing to a lower egg production (*cf.* below).

There is a clear relationship between the number of BSF adults introduced every week in the insectarium and the amount of eggs that were collected (Graph 4-2). However, several other factors, non considered or measured here, can also influence egg production.



Physiological factors (low fecundity, infertility, nutritional deficiencies), behavioural factors (low mating frequency, poor identification of egg-laying sites, predation), abiotic factors (lack of environmental stimuli) or technical factors (egg laying occurring out of nest boxes, efficiency of egg collection) might impact the results. If some of these parameters could be better controlled in the future, the egg yield could be much higher.

Impact of Unbalanced Sex Ratio on the Production

There is no account of skewed sex ratio of BSF populations in the scientific literature, in contrast to the situation observed in the insectarium at Depok (64% of males and 36% of females). Tomberlin *et al.,* (2002) showed that the origin of a BSF population (wild or reared in captivity) and the diet used to feed their larvae did not affect the sex ratio in this species. We have attempted to evaluate the production loss caused by the deviation of the sex ratio in the insectarium at Depok. With an average of 464 000 BSF emerging in the pilot-insectarium and a balanced sex ratio (1:1), the amount of eggs produced every week should reach about 4.2 kg (with an average number of 637 eggs per female; Rachmawati, 2010). In Depok's insectarium, only 36% of emergent insects are females (thus 167 000 individuals), which can account for a reduction of egg production by a margin of 28 %. Nevertheless, the actual egg production (215 g per week) is far from the 3 kg that would have been expected with the observed sex ratio.

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras
 ISBN: 978-979-493-610-8

Chapter 5

Production of pupae

Emilie Devic[(a)], I Wayan Subamia[(b)], Pascale Talamond[(a)], Domenico Caruso[(a)],

(a) UMR226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan No. 13, Pancoran Mas, 16432 Depok, Jawa Barat, Indonesia.

The production of adult BSF is a continuous process so there must be no interruption otherwise the entire production cycle is compromised. Consequently, a cohort of pupae that are homogeneous in age has to be transferred every week in the insectarium. Special care is needed during larval development and metamorphosis (continuous supply of high-quality food, careful handling of pupae, etc.) because adults do not feed, so their fitness largely depends on the food during their larval period. The higher the energy reserve accumulated by the larva, the higher the fecundity and the longer the life of the adult (Tomberlin *et al.,* 2002).

Growth in Captivity: from Larva to Imago

The production pilot operates as a "closed-loop process". Generations of insects are produced one after another without any introduction of wild individuals. The large amount of individuals produced by generation allows enough genetic mixing and reduces risks of consanguinity.

To optimize the development of larvae, it is necessary to supply them with a specific diet that will fuel a fast growth and enable the accumulation of lipids. These lipid resources will represent the largest part of the energy used by adult insects, and are just essential for performing oogenesis and producing high-quality eggs. The growth substrate is not only food for the larvae; it is also their living environment.

Temperature, humidity, granulometry and thickness of the substrate layer are key parameters for larval development. From hatching onwards, larvae are reared at 25-38°C on anaerobically degraded PKM. They begin feeding from the first day and display an exponential growth rate during the first eight days of life. Then, growth rate decreases and 18 days after hatching (dah), the insect has reached the pre-pupal stage: its size and weight are maximal, about 20 mm and 180 mg, thus about 24 times as long and 9,000 times as heavy than at hatching.

Body size (length and width) will remain unchanged until the emergence of the imago. By contrast, body weight progressively decreases because of

both cessation of feeding and use of reserves for metamorphosis. Adults have a much lower body weight than pupae (less than 40 mg *versus* more than 150 mg). Most reserves stored during larval development have been used for metamorphosis and the exuvia is about 10% of the total weight of the pupa (Tomberlin *et al.,* 2002). Moreover, males are significantly smaller than females (Table 5-1).

Table 5-1 Differences between-sex size and weight dimorphism of BSF in the insectarium at Depok

Gender	Body Length (mm)	Body Weight (mg)
Male	12.7 ± 1.1	30.2 ± 1.2
Female	13.5 ± 1.4	37.9 ± 1.6

In routine production, larvae of BSF reach the pupal stage within 28 ± 3 days. From then on, about 14 days are necessary to allow the emergence of 90% of the cohort (Cf. Chapter 4).The life of adult BSF is 8-9 days and oviposition starts generally 4 days after emergence (Rachmawati, 2010).

The development of a cohort (from egg collection to egg laying) thus lasts about 46 days. This duration differs from those reported by other authors (May, 1961; Tingle *et al.,* 1975 in Tomberlin *et al.,* 2002). Environmental factors such as temperature or humidity can influence the length of a cycle. In the production pilot at Depok, these factors are not regulated, and this may contribute to increasing the observed variability.

Hatching and the First Six Days

Every week, a small proportion of eggs collected in the insectarium is weighed carefully to determine both the hatching rate and the survival rate at 6 dah (as 6 day-old larvae are large enough to be counted accurately). Eggs are placed on a fine wire mesh (1 mm) and stored in an incubator (plastic container) filled with 2-5 kg (humid weight) of anaerobic degraded PKM. Small larvae will grow in this container during the first 6 days of their life (Figure 5-1).

Figure 5-1 Rearing of larvae from 0 to 6 days after hatching –Left: incubator with PKM and eggs ready to hatch placed on a small grid. Right: hatching; the small larvae are visible to the naked eye. The yellowish masses are eggs

The hatching rate, as determined on the basis of survival at 6 dah, is 79.5 ± 18.8% in the pilot conditions. This parameter is estimated for every production cycle, because it is necessary to evaluate and optimize the various stages of the production system (pupae, larvae used as live food). In order to measure it, one should weigh the whole content of the plastic container (PKM + larvae) then mix the substrate carefully (using a spatula, for example) to make it as homogeneous as possible. The next step is to collect a sample of about 20 g that will be weighed accurately.

This process is repeated three times. Finally, larvae are isolated from the substrate and counted. The mean of the three samples gives an estimate of the total number of larvae in the plastic container. The variability of survival and hatching rates depends mainly on the "quality" of eggs; but under the production conditions at Depok, the initial egg quality was not assessed directly as no indicator had been defined.

However, to improve performance, some rules of good rearing practices can be suggested: hygiene of incubators, quality and quantity of supplied PKM, constant and optimum hygrometry (<60%), ventilation of incubators on warm days, preventing eggs of flies or other insects from being laid in the incubators, etc.

Production of Pupae

Step 1: Weigh the dry PKM and load the digester

Step 2: Spread dry PKM in the digester with a rake, and add water and mix progressively

Step 3: Spread further the PKM soaked in water to create an homogeneous layer in the digester (about 5 cm thick)

Step 4: Cover the digester with a mosquito net

Figure 5-2 Loading steps of a digester with PKM

Production of pupae

Pupa production is performed in the larvarium. The production rates given in this chapter are derived from the analysis of 26 experimental cohorts. Rearing is performed using 5 circular digesters (used surface: 8 m^2 for a maximal usable surface of 16 m^2). The digesters are used successively, with a weekly rotation. Every week, a digester is filled with 164 ± 2 kg of PKM and 330 ± 13 L of water to initiate aerobic degradation. This is a 6-day long step, the digester being covered by a mosquito net to prevent any other animal to get into PKM (Figure 5-2).

At the same time, when the digester is loaded with PKM, 22 g of eggs (collected in the insectarium) are placed inside an incubator. As each gram of eggs is made of more than 33.600 larvae and as the mean survival rate at 6 dah is 80%, on average 585.300 ± 44.500 larvae are introduced in the digester. Then PKM is arranged in a 5 cm thick layer that should be saturated in humidity (Figure 5-3).

Figure 5-3 Digester with 6-day old BSF larvae (dark spots). A layer of moulds and other micro-organisms covers PKM (white and green spots)

The constant ploughing by larvae, the high temperature (24-48°C) and sometimes the low humidity (<20%) inside the larvarium lead to the dehydration of PKM. Hence, as long as larvae continue feeding (until

about the age of 16 dah); It is necessary to add some water in the digester from times to times in order to humidify the substrate and to make sure the temperature will not be excessive.

About 135 ± 30 L of water are necessary during this period of the production, so it is better adding a small amount of water (20-50 L) in the digester each time the substrate becomes too dry.

The behaviour of BSF larvae can be a good indicator of humidity and temperature. If temperature is too high or the substrate is too dry, larvae tend to move up to the surface of the substrate. If there is too much water, they tend to leave the substrate by spreading on the walls of the digester (their wet body enabling them to stick onto vertical surfaces).

Colonization of Substrate by Other Species and Competition

Colonization of PKM by other species (mostly *Musca spp*.) has to be prevented for several reasons: first, these harmful species have most often a faster larval development than BSF, so they will reproduce faster within the larvarium and they will become overabundant. Their increasing number will complicate the task of operators (annoyance and additional handling). It also brings an additional sanitary risk, as these species – unlike BSF - are often responsible for the spreading of pathogenic agents. Moreover, the larvae of these insects can compete with BSF for food and therefore interfere with the whole production. PKM colonization by other flies larvae in Depok pilot was not excessive and generally it has been observed that competition turned in favour of BSF. Indeed, it has been experimentally demonstrated that when the substrate is colonized by a large amount of BSF larvae, flies of the dipteran family Muscidae could hardly lay their eggs, as BSF larvae release a volatile compound that acts as an allomone and inhibits oviposition (Furman *et al.,* 1959; Axtell and Edwards, 1970; Sheppard, 1983; Bradley and Sheppard, 1984). In the pilot, if colonization by *Musca spp.* occurs in spite of the protection net covering the digester, a large amount of water is added to drown them. Indeed, BSF larvae resist flooding much better than these harmful species. However, it must be reminded that flooding can slow down the growth of BSF larvae and can be deadly for prepupae and pupae. Observations show that PKM colonization by other larvae generally occurs at an early stage in the production cycle, between the loading of the digester with PKM and the introduction of 6-day old BSF larvae.

These behavioural criteria are reliable but a regular monitoring using temperature and hygrometric sensors is recommended in order to act before environmental conditions become unfavourable. The last week of the cycle is dedicated to pupation. Larvae become pre-pupae, their colour turns to dark brown, and they gather along the walls of digesters.

This "migration" displaces residues of PKM as well as already formed (motionless) pupae towards the centre of the digester (Figure 5-4).

Figure 5-4 Digester during pupation of BSF larvae (23-28 dah). Brown/black pre-pupae (left) shoal together along the walls. Their movements progressively push residues of PKM, already formed pupae and exuviae towards the centre of the digester

The survival rate between the introduction of larvae and the collection of pupae (from 6 to 28 dah) is estimated from the total weight of collected pupae and their average body weight. On average, 76 ± 12% of BSF larvae reach the pupal stage.

It has to be noted that cohorts produced in the pilot at Depok display a sex ratio in favour of males. Sex differentiation is a complex process that takes place during larval development (*cf.* below "Sex Determination in Insects").

In synthesis, the unbalanced sex ratio of BSF observed in Depok can have various origins, the contributions of which still have to be elucidated. However, in view of the very high temperatures (sometimes over 40°C) in the fermenting substrates during the larval stage of BSF, the role of this factor on deviations of the sex ratio and also possibly on the sterility of adult BSF, should be examined in priority.

Sex Determination in Insects

The deviation of the sex ratio of *H. illucens* with a predominance of male individuals, as observed in the production pilot at Depok, has never been reported in scientific literature. By contrast, Tomberlin *et al.,* (2002) have noticed a higher percentage of emergent females (55.2 - 60.5%) produced on 4 different substrates (no significant differences between substrates). The sex ratio of insects is often unbalanced, for different reasons. In holometabolous insects (Coleoptera, Diptera, Lepidoptera, Hymenoptera, ...), the nymph transforms into an adult at the pupal stage. However, the sex of the insect has been genetically determined long before this stage, mainly through the presence of hetero-chromosomes (XY or XO in males and XX in females). In dipterans, sex determination is further influenced by secondary factors carried by autosomes that modulate in a cascading pattern the expression of various genes. With regard to drosophilae, if X: A ratio is 1:1 (2X: 2A), the cascading expression of these genes (*Sxl*→*tra*→*dsxF*) leads to a female phenotype (Saccone *et al.,* 2002). This process is not shared by other dipterans. However, in spite of differences, the basic strategy of sexual differentiation in dipterans remains similar: a primary genetic signal (different for males and females), a key gene that responds to the primary signal and a gene that acts like a master switch and eventually determines the phenotypic sex of the animal (Schütt and Nöthiger, 2000). For example, with regard to *Musca domestica*, the predominance of males can be linked to determining factors of male sex (M) on autosomal chromosomes (Hamm and Scott, 2009). In some dipterans such as the drosophila, deviations of the sex ratio can be mediated by *tra2,* a temperature sensitive allele. When drosophilae are carrying this mutation, sexual determination depends on temperature. If temperature is 16°C, they develop into fertile females, whereas a temperature of 29°C results in the development of sterile males (Belote and Baker, 1982). The effect of temperature

on sex determination is not systematic in insects. For example, in *Cochliomyia macellaria*, no deviation of the sex ratio was observed at temperatures ranging from 20 to 28°C (Boatright and Tomberlin, 2010). Parameters other than temperature, such as consanguinity, can act on sex determination. In the hymenopteran *Diprion pini,* the proportion of males in a progeny increases significantly when there is a strong degree of consanguinity (Géri *et al.,* 1995). Other factors, of nutritional nature, have also been reported to act on sex determination.

Operations of Sorting, Harvest and Transfer

The proportion of pupae is a good indicator of whether the production cycle is over. If it is less than 60%, then the harvest should be postponed for a few days. If it exceeds 80%, partial or full harvest can be performed (some pre-pupae can be put apart for a few days until they become pupae). The harvest is a time consuming operation that requires two operators for at least half a day. The first step is to separate pupae from digestion residues, exuviae, pre-pupae and late larvae.

Sorting has to be done by hand and the content of the digester is progressively emptied. A highly technical and traditional movement allows residues of PKM and small larvae to go through the fine mesh of a bamboo fibre basket. Lighter exuviae fly off and only pupae remain in the sieve (Figure 5-5). It might be possible to mechanize the sorting process, but metamorphosis is a delicate stage and it matters that pupae be processed adequately so as not to hurt the developing adult.

Therefore, future BSF producers have to remind that rearing practices and environmental conditions (mainly temperature) can affect the sex ratio of cohorts.

Figure 5-5 Sorting of BSF pupae using a bamboo basket with traditional movement

Other methods can be used to sort out larvae from the growth substrate. For example, as larvae are searching for dry areas to pupate, one can take advantage of this behaviour to design an "auto-collecting" method. Consequently, Diener *et al.,* (1983) proposed digesters with oblique walls that enable pre-pupae to extract themselves from the substrate. Then, they get into plastic tubes leading them towards collection containers. This method has also been used by other authors (Sheppard, 1992; Newton *et al*., 2005a). Olivier, (2004) patented a digester of organic waste using BSF larvae and their auto-collecting capacity.

As soon as the pupae are sorted out, they are either stored for further processing or transferred into the puparium of the insectarium for broodstock renewal. For the latter operation, a few kilograms of pupae are put into a clean plastic container. This has to be done very carefully because pupae are highly sensitive: strong shocks or hypoxia (that may occur if the storage in the container is too long) will have a negative impact on their development and on the proportion of emerging adults. Finally, digestion residues of the substrate are stored in the bags that initially contained PKM whereas rebuff larvae (late larvae or dead pre-pupae) are composted.

Productivity

A digester is typically used during 28 ± 3 days (from PKM loading to pupa collection). On average, 44 ± 17 kg of BSF pupae (wet weight, thus about 5 kg/m^2, or 585 000 ± 9 100 individuals) are produced every week from 164 ± 2 kg of PKM (dry weight) and from about 22 g of eggs. Pupae and co-products (bio-fertilizer, exuviae) represent a total production of 130 kg. In other words, every kg of dry PKM produces 0.266 kg (wet weight) of pupae (a FCR of 3.76) and about 0.52 kg of co-products. Digestion residues represent 56% of bioconversion products *versus* only 34% for BSF pupae. Rebuff larvae (late or dead larvae) amount to a significant (10%) proportion of the initial substrate weight (Figure 5-6).

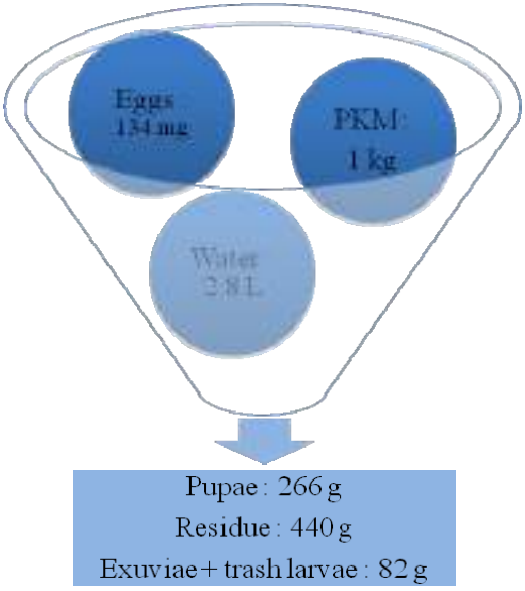


Figure 5-6 Inputs and outputs from the section "larvarium" of the Depok's pilot for 1 kilogram of dry PKM

Several hazards can jeopardize the productions. Diener *et al.,* (1983) have noted that high concentrations of heavy metals such as zinc are lethal for

BSF larvae. In case of high or frequent mortality, the contamination of the substrate by these compounds should thus be considered.

A biological enemy of *Hermetia illucens* is also well known, namely *Trichopria sp.* (Hymenoptera: Diapriidae), which is an endophage-parasitoid of their pupae. Bradley *et al.,* (1984) reported a prevalence of up to 32% of *Trichopria sp.* towards BSF pupae in Georgia (USA). They claimed that *Trichopria sp.* had never been observed in pupae of common flies. In the pilot at Depok, the occurrence of this parasitoid was reported (Rachmawati, 2010; Figure 5-7). The propagation of this parasitoid was prevented by covering the digesters with mosquito net during pupation.

Figure 5-7 Parasitoid of BSF, Trichopria sp. a) The adult; b) A pupa of BSF after emergence of parasitoids (note the small holes in the exuvia) and c) Larvae of the parasitoid developing inside the BSF pupa

Co-products of Bioconversion

The rearing of BSF larvae produces residues originating from the digestion of substrate. These residues display characteristics of bio-fertilizers and are similar to compost. The first experiments by Newton *et al.,* (2005a) with residues of BSF larvae digestion that were used on basil *(Ocimum basilicum)* and sorghum *(Sorghum sudanense)* produced little encouraging results, but BSF larvae had grown on liquid pig manure. By contrast, residues of PKM (the main characteristics of which are: C/N = 5.8; N=3.0%; P=0.82%; K=1.04% and C=15.7%) have already proved their effectiveness as bio-fertilizer. For example, the growth of *Vigna unguiculata sesquipedalis* (a leguminous vegetable cultivated in Asia) was at least 4 times higher when a PKM residue was added as fertilizer (Anggraeni, 2010). Consequently, this co-product is truly worth collecting and it is further abundant. In the Depok pilot, these residues represent 44% of the PKM used.

In the same way, exuviae are of interest. They are less abundant than PKM residues, but they nevertheless amount to about 10% of pupae weight. Exuviae are valuable as they are rich in chitin (one of the main component of the arthropods exoskeleton). This molecule has an unquestionable commercial interest due to its high nitrogen content (6.9%) compared to synthetic cellulose (1.25%). It is extensively used for cosmetics, medical purposes, agri-food, wastewater treatment, etc. However, the technical and economic feasibility of chitin extraction has not been assessed yet (Diener *et al.,* 2011).

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF)
Hermetia illucens, Stratiomyidae.
Editors : Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras

Chapter 6

Rearing of fattening larvae

Domenico Caruso[(a)], Rina Hirnawati[(b)], Emilie Devic[(a)], Melta Rini Fahmi[(b)] and Pascale Talamond[(a)]

(a) UMR226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan No. 13, Pancoran Mas, 16432 Depok, Jawa Barat, Indonesia.

BSF rearing has several positive outputs: the reduction of wastes or any organic by-products, the production of pupae for broodstock renewal and the production of larvae dedicated to animal feeding. If the main aim is the production of larvae, it is important that the substrate's composition results in a high survival rate, a fast growth and a good nutritional value of BSF larvae for the targeted animal species. As for every rearing activity, it is also necessary to optimize the feeding level (ration), which is a key parameter of economic profitability. Knowledge on the nutritional requirements of BSF larvae is still limited.

Advances in this field will be a challenge for researchers and a premium for future producers in order to select the right substrates and optimize the production. Two types of larvae can be produced:

- Mini-larvae, used as food for small ornamental fish, fingerlings or pets (*cf.*"Mini-larvae production");
- Fully grown larvae, which can reach a maximal weight of 100 mg, approximately and which are either used as live food or processed into a maggot meal (feed ingredient).

Mini-Larvae Production

Hem and Fahmi's patent (WO / 2009 / 136057A2) describe the method used to produce BSF mini-larvae. The main characteristic of these larvae is their small size: their body diameter varies between 1 to 4 mm and their body length ranges from 5 to 12 mm. The amount of substrate is adjusted to limit the growth of the larvae depending on the size desired. Eggs are incubated during 1 to 3 days at a temperature of 24 to 35°C over a substrate, made of cereals or oil cakes following an aerobic or anaerobic degradation process during one week. Then, larvae are grown during about 10 days on the selected substrate, before being collected and cleaned. The patent also describes how to preserve live larvae at the same size as at the time of collection: just after being collected, BSF larvae are stored in a cool (15°C, 70 % humidity) and dark environment, which causes them to enter in diapause (i.e. reduction of physiological activity).

Growth and Conversion Rate of Fully Grown Larvae

The growth performance of BSF larvae used in animal feed has been investigated in the course of several dedicated experiments and mass production trials.

Experiments aimed at characterizing the growth and other zootechnical performances of BSF larvae depending on the food ration. Mass production trials aimed at testing whether these results could be upscaled to larger productions.

The terminology used to describe the zootechnical performances of the larvae stands as:

Food Conversion Rate (FCR) = $R_{distrib}$/ B_f – B_i

Specific Growth Rate (SGR) (% WW[1] per day) = ((ln B_f - ln B_i) / D) * 100 Residues rate (%) = 100 * amount of residues (WM) /amount of substrate (WM) With: $R_{distrib}$: the total weight of the ration supplied (dry mass, DM); B_f : the final biomass harvested (wet mass, WM) and B_i: initial biomass (WM); D: number of rearing days.

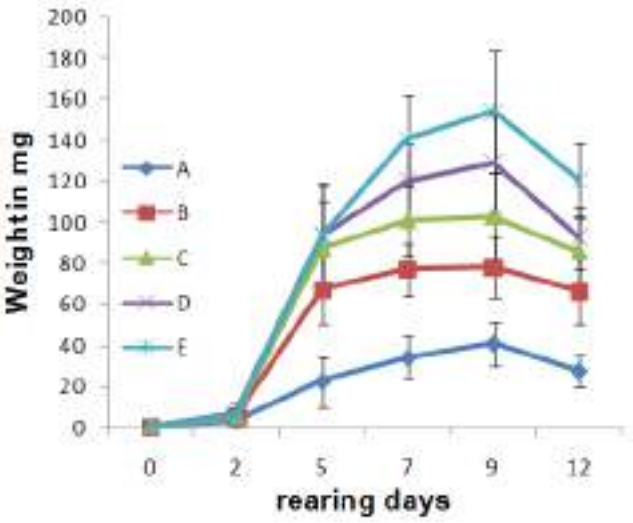
Five groups of BSF larvae (1000 individuals/group, 6 dah initially 0.7 mg weights) were transferred onto a bed of PKM (anaerobically degraded) and reared during 12 days. The food ration supplied to the first group was 4.0 mg of PKM, dry matter (DM) per day and per BSF larva; other daily rations were calculated using a 2-log scale geometric progression (i.e. 4, 8, 16, 32 and 64 mg of PKM per day and per larvae).

On the first day of the experimentation, a unique supply provided the entire food ration for the 12-day long rearing period.

1 Wet Weight, WW

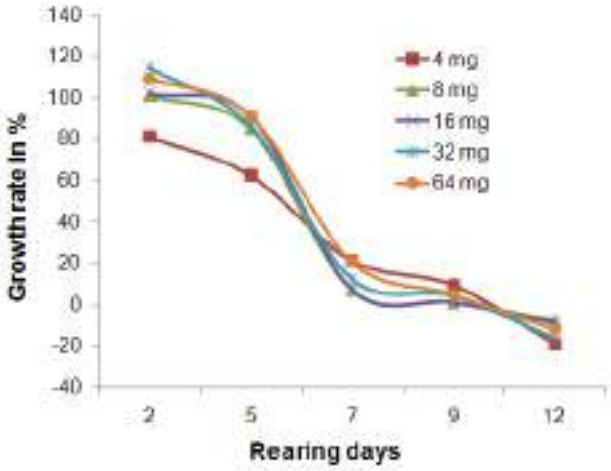
Samples of BSF larvae were collected after 2, 5, 7, 9 and 12 rearing days to measure growth. The growth trajectories of BSF larvae fed different food rations vary very little (Graphic 6-1), except for the lowest food ration (4.0 mg per larva per day).

However, the body weights of BSF larvae differ strongly depending on the daily food ration: the highest median weight (153 mg) has been observed for larvae fed the highest feeding level (64 mg PKM DM per day and BSF larva). SGR decreases slightly with increasing age and body size until the fifth rearing day (equivalent to 11 dah[2]) then, it abruptly decreases until the ninth rearing day (15 dah). Beyond this age, the SGR becomes negative whatever the feeding level (Graphic 6-2).Other results are summarized in table 6-1.



Graph 6-1 Individual weight of BSF larve (in mg) according experimental food ration. Values are means ± standard deviation (n 480 for each group)

2 Days After Hatching



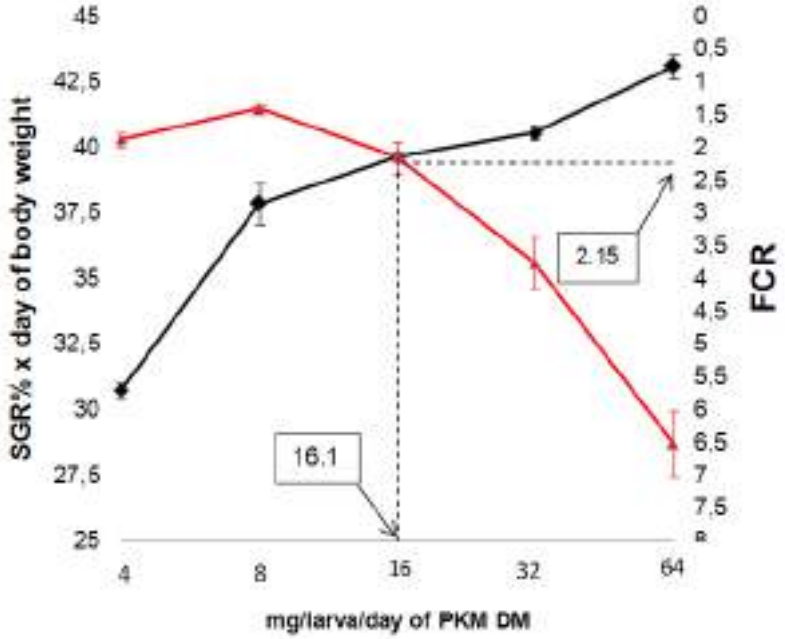
Graph 6-2 **Specific growth rate (SGR, %) of BSF larvae depending on food rationing (mg dry PKM per larva and per day). Data are means of three replications**

Table 6-1 **Effect of PKM rationing on the performances of BSF larvae from 6 to 18 days after hatching. Values are means ± standard deviations of 3 replications. Within each column, letters A to E indicate the different levels of rationing. Asterisks indicate statistical differences: *: a < 0.05; ** a < 0.01; *** a < 0.001. One way Anova, Tukey post hoc tests**

Food Ration (mg day^{-1} Larva^{-1})	Yield (g)	FCR	Survival (%)	Wet residual (%)
A) 4.0	25.7 ± 1.54 (ED ***; C **)	1.88 ± 0.01	95.5 ± 1.9	19.8 ± 0.4 (CE **; D *)
B) 8.0	51.4 ± 25.91 (E ***; CD **)	2.47 ± 1.75	93.6±3.9	24.6 ± 1.2
C) 16.1	90.3 ± 10.11 (A **; B *)	2.16 ± 0.26	77.6±0.7 (ABDE ***)	27. 5 ± 1.1
D) 32.2	103.4± 10.26	3.77 ± 0.39	96.7±2.3	25.6 ± 3.0
E) 64.4	119 ± 9.15	6.52 ± 0.51 (ABC ***; D **)	95.3±2.0	28.1 ± 3.1

For the producer, maximal growth is not systematically the main objective. If the substrate is rare or expensive, food conversion is the true priority.

The best compromise between SGR and FCR is a feeding level of about 16 mg of dry PKM per day and per larva, which allows a SGR of 39.5 % day^{-1} with a FCR of 2.15 (Graphic 6-3).



Graph 6-3 Averages Specific Growth Rate (SGR) and Food Conversion Rate (FCR) of BSF larvae with 5 feeding levels. The bold plain dark curve is the average SGR (%) calculated from the three replications (represented by the black dots). The red curve indicates variations of FCR with increasing feeding levels. Data are expressed as average and error bars are SEM. The hatched projection lines on the graph axes indicate the best compromise between SGR and FCR

According to these results, in mass production conditions, if the producer starts his production using 10 g of BSF eggs and with an average daily ration of 16 mg of dry PKM per larva; he could expect a final biomass of

approximately 28 kg of fresh BSF larvae. In other words his productivity would be 466 g of larvae per kg of dry PKM (taking into account a mortality rate of 30%).

These experimental results were confirmed under conditions of mass production where similar performances were obtained, with a production of 477 ± 42 g of fresh BSF larvae per kg of dry PKM, a FCR of 2.18 ± 0.32 and an estimated survival rate of 64.0 ± 13.4 %. However, under mass production conditions, it took no less than 14 days before BSF larvae attained the target weight of 100 mg *versus* 9 days under experimental conditions.

The mass production trials were performed starting with 6-day old BSF larvae, however with an initial body weight much smaller than the larvae used for the production of previous experiments (about 0.3 mg). Small larvae might be more sensitive to weighing, transportation and mixing with PKM; this could result in higher mortality and slower growth during the first rearing days. Moreover, the amount of residues of PKM in mass production trials (45.0 ± 2.5 % of PKM residues) was higher than during small scale experiments (see table 6-1).

In other words, PKM in large scale trials was not used as extensively as during small scale experiments, thereby suggesting that food availabity was not the sole limiting factor behind the slower growth of BSF larvae in these circumstances. However, PKM is not only a food resource for BSF larvae, it is also their habitat. Uncontrolled variations in PKM degradation, hygrometry or temperature (of the substrate itself), can impact the growth and survival of BSF larvae.

The rearing density (number of larvae per unit of surface) can also influence the growth or survival rates of BSF larvae. Several experiments suggest that BSF larvae can cope with densities up to 8 larvae cm^{-2} without any significant effect on their survival rate.

In other words, the polyphagy of BSF larvae – thanks to their specific bacterial symbiotic flora - allows the use of several types of feed of

different natures (Jeon *et al.,* 2011). A protein content of 15 % in the substrate is often recommended for the growth of BSF larvae (Tomberlin and Sheppard, 2002; Myers *et al.,* 2008). This is the case of PKM.

However, a satisfactory growth can also be achieved from substrates with a lower protein content but with digestible carbohydrates and lipids (e.g. copra residue with 6 % proteins). Digestible carbohydrates are major energy sources for insects, but insects can also synthesize them from lipids and amino acids (Genç, 2006). Moreover, if the raw substrate partially or totally is poor in such carbohydrates, they can become available following a pre-degradation process (aerobic or anaerobic).

The lipid content of the substrate is not a major criterion for substrate selection. Yet, metamorphosis and reproduction of BSF adults strongly depend on lipids, but their larvae and substrate microflora are able to synthesize them from other molecules.

The BSF larva is a bacteriophage, therefore it can also gain energy while feeding on the bacteriae colonizing the substrate (Erickson *et al.,* 2004; Sealey *et al.,* 2011, Yu *et al.*, 2011). However this resource is hardly measurable. In synthesis, BSF larvae present a considerable trophic plasticity and can use a broad range of substrates. Nevertheless, knowledge on their nutritional requirements remains poor regarding both basic nutriments (vitamins, minerals, amino acids) and potential anti-nutritional compounds present in substrates.

Growth of BSF Larvae on Substrates Other than PKM

The growth of BSF larvae varies significantly depending on the rearing substrate and its characteristics (nutritional, physical, etc.). Tomberlin *et al*., (2002) did not observe any significant difference between the body weights of BSF pre-pupae (153 - 171 mg, wet body weight) and the time needed for their development (22.5 – 24.1 dah) while using three different and highly nutritional diets for insects. Nevertheless, zootechnical performances differences can be different when the substrate is made of waste or of agricultural by-products. According to

Diener *et al.,* (2009), the higher the feed availability (results observed using poultry feed) the faster larvae reach the prepupal stage with a higher individual body weight. They estimated that when using such a diet, a daily feeding rate of 100 mg of feed per larvae (60 % moisture) is the best compromise between the biomass produced (a BSF prepupa weighting 48 mg DM at 16 dah, 60 % moisture) and the substrate reduction (42 %). Myers *et al.,* (2008) who have used dairy manure, confirmed these results: when BSF larvae are fed a daily ration of 70 g of manure, they reach the prepupal stage within 43 days, with a wet body weight of 137 mg. These larvae are about 35% heavier than those fed with 27 g manure per day. However, with the highest manure ration, the substrate reduction rate of the substrate was 25% lower than with the lowest ration.

The substrate composition is a key parameter: a mixture of faecal sludge and market wastes (ratio 1:1) produces a faster growth rate (at 18 dah) and a higher average individual body weight of prepupae than when faecal sludge is used alone (respectively 67 and 55 mg dry weight; Diener *et al.,* 2011). St Hilaire *et al.,* (2007) also recommend mixing cattle manure with fish wastes for a better growth of BSF larvae. Inoculating bacterial strains (of *Bacillus* sp.) in poultry manure might also improves the growth of BSF larvae (Yu *et al.,* 2011). At Depok, experiments performed using other vegetal substrates (water spinach, water hyacinth, taro leaves, copra residues, etc.) produced variable growth rates of BSF larvae.

Cleaning, Collection and Transformation

If food has been rationed properly, only BSF larvae and bioconversion residues should remain at the end of production cycle. Nevertheless, the substrate residues can still be humid by then, hence sorting can be more difficult.

At this stage, BSF larvae have stopped growing and should be homogeneous in size, so they can be sorted with a sieve. Small amounts of larvae can be cleaned with water, and residues of bioconversion pass through the sieve mesh (Figure 6-1).

Figure 6-1 Washing BSF larvae at the end of the production cycle. A bamboo sieve is used to separate the larvae from the substrate residues

Once larvae have been cleaned and graded, they are either kept alive at 4°C (they enter in physiological dormancy) or slaughtered. Slaughter can be performed by drying or freezing. If money is short and energy savings are a premium, larvae can be sun dried in bamboo baskets as long as air circulation suffices. (Figure 6-2).

Figure 6-2 Bamboo baskets used for sun drying BSF larvae

The drying process of larvae (with an efficiency of about 95%) requires 17 hours of sunshine under the following conditions: light intensity >20 000 Lux, temperature of 38 ± 4°C and air humidity of 47 ± 6%. The weight

loss of BSF larvae following drying is estimated at 65% of their wet body weight Alternately, it is possible drying them using a homemade oven using a small electric heater and a closed wooden structure (Figure 6-3).

Figure 6-3 Hand-made oven used in Depok; a) overview; b) inside view

For larger amounts of larvae, mechanized collection is necessary.

Mechanization of Collection Process

The collection of BSF larvae and the separation from residues are tedious and time-consuming tasks. It is also critical for pupae. Mechanical sorting is not common in small-scale or pilot productions; however, it might be necessary in order to optimize the collection efficiency, mainly for fully grown larvae or to sort larvae of different sizes (calibration process) for commercial purposes. A sorting machine made of a rotating drum and an electric motor has been developed and experimented in the production pilot at Depok (Figure 6-4). The drum comprises several metallic cylinders equipped with slots of increasing dimensions, the whole being slightly tilted in order that the speed at which larvae progress from the entrance to the end of the machine is not excessively slow or rapid. Larvae are loaded through a large opening allowing then they fall down into successive cylinders more or less rapidly. As they go through the slots corresponding to their size range, they fall into baskets placed underneath. The system is efficient for grading larvae, but not for pupae as physical shocks can disturb their development and metamorphosis. Its performance also depends on the efficiency of the preliminary cleaning process, and t he operation has to be repeated several times before all larvae are sorted properly. After repeated trials, this practice has been

abandoned because it was less efficient than the manual grading using bamboo sieves with different mesh sizes. The faster the sorting process, the higher its efficiency. Indeed, the constant wriggling behaviour of BSF larvae and their body elasticity facilitate their outlet through mesh sizes smaller than expected, thereby compromising the overall efficiency of the sorting process. Whatever the method (manual or mechanised) used for size grading, BSF larvae have to be cleaned and water has to be removed before sorting.

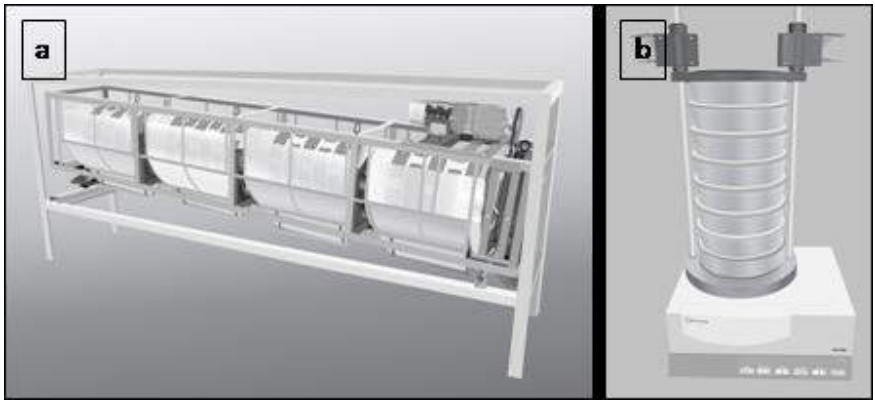


Figure 6-4 Mechanization of BSF collection and calibration: a) machine with rotating drum and b) sieving over a collecting basket

Once the drying process is completed, BSF larvae can easily be processed into a fish-meal. Alternatively BSF larvae can be slaughtered following the exposure to subzero temperatures. Small amounts of larvae are disposed in small bags and stored at -18°C for several hours. They die rapidly and can be preserved at the same temperature throughout.

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras
 ISBN: 978-979-493-610-8

Chapter 7

Ecological Intensification of Aquaculture: Perspectives offered by BSF

Domenico Caruso[(a)], Sawung Cindelaras[(b)] and Emilie Devic[(a)]

(a) UMR 226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan No. 13, Pancoran Mas, 16432 Depok, Jawa Barat, Indonesia

Aquaculture production in Southeast Asia, and especially in Indonesia, comes from small farms where producers are often involved in other agricultural or commercial activities that strengthen their economic resilience. However, small scale aquaculture is under economic pressure and competition with commercial aquaculture or imported products that often cause producers to intensify their production. As often stated in the present work, availability and cost of animal proteins are the main bottleneck to the improvement of fish farm productivity. Most intensification attempts in small fish farms failed due to insufficient food supply or high production costs, resulting in an increasing impoverishment of farmers and sometimes in environmental impacts.

Production, Intensification and Ecologically Intensified Aquaculture (EIA).

The intensification of aquaculture productions, as for those of agriculture, relies on the increase of inputs (food, energy, densities, etc.). It can be described as "transformation agriculture" with an intensification gradient. In this productive concept, the ecological component of the system is largely forgotten and the rearing environment (animal boxes or raceway, for example) merely represents no more than a physical support for production. This conventional intensification process requires an increasing number of production factors per unit of production area, as well as higher cash flow and management skills, which are rarely met in rural fish farms. Ecologically Intensified Agriculture (EIA) is a new paradigm that offers an alternative way for rethinking the production of biological organisms. It aims at using ecosystem resources and mechanisms for optimizing production while taking into account all aspects of sustainability, including potential or actual social, political, economic and environmental impacts. Moreover, ecosystem intensification further aims at preserving rural activities without disruption in the agricultural web and it can be an opportunity for small fish farmers to improve their living standards.

The production of BSF larvae can provide small fish farms with significant amounts of animal proteins which farmers could not afford with other protein sources. However, BSF production in its current form remains poorly adapted to the context of small fish farms. Indeed, it requires a significant initial investment and the continuous supply of production substrate (here PKM), which can be compromised by low treasury and logistic reasons. Producers indeed have to pay for PKM (and most other agricultural by-products, such as copra residues) as well as for transport, whereas their cash flow is generally low. Furthermore, the supply of these products can be irregular, because of fluctuations in availability or difficult transport during the rainy season, whereas a continuous supply of substrate is just crucial for BSF larvae production and renewal of BSF broodstock.

An alternative to this issue consists in using locally available growth substrates for BSF larvae, which can be produced in an agro-fish-farming ecosystem, known as "ecosystem resources". In a broader context, the use of ecosystem resources can be an alternative to the intensification of productions in agriculture and aquaculture (*cf. "*Production, Intensification and Ecologically Intensified Aquaculture, EIA"). In regard to aquaculture, the use of ecosystem resources is already a common practice in many extensive productions. However, its regular use in intensified productions requires sophisticated biological engineering (multi-trophic ponds, organic fertilization) that complicates the management of production environments by fish farmers. In Indonesia, the vegetal biomass in traditional fish farms is generally abundant, but its interest has not been wisely taken into account until recently, because no efficient "bioconverter" had been identified or validated (Figure 7-1).

BSF larvae, because of their capacities to use a broad range of substrates, offer sustainable solutions for providing alternative sources of animal proteins in a rural environment. Dedicated experiments have been carried out in order to test for the relevance of this hypothesis.

Figure 7-1 Vegetal diversity around traditional fish farms of giant gouramy in Sunda (western Java). The colonization of the fish rearing pond by water hyacinth can be seen in the foreground

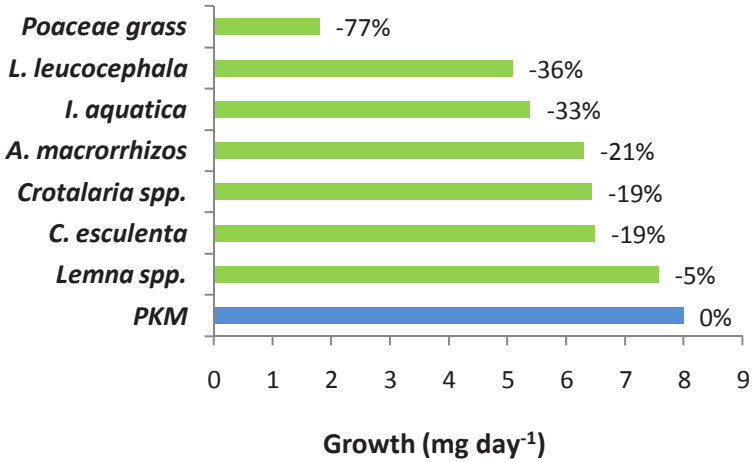
"Ecosystemic" Plants and BSF rearing

Vegetal diversity and exuberance, in and around Indonesian fish farms is particularly rich, even if their biomasses still remain to be determined. In Depok, several plants that were abundant around production ponds or were known to have a high nutritional value, were experimented as growth substrates for BSF larvae, and compared with PKM. Information on these plants is given in table 7-1. Some of these species, such as the water hyacinth *(Eichhornia crassiceps)* and the water spinach *(Ipomoea aquatica)*, are invasive species. For experiments, selected plants were first cut, crushed with a craft food chopper (Figure 7-2) then stored in a closed

plastic container for one week, so to produce a semi-aerobic microbial degradation. These substrates were given to 6-day old BSF larvae (cf. chapter 6).

Figure 7-2 Small chopper used to crush plants into growth substrate for BSF larvae

The first degradation allows usually a volumetric decrease, a homogenization of vegetal biomass and the separation of aqueous fraction for the least lignified plants (*Ipomoea aquatica*, *Colocasia esculenta*). After one week of degradation, most of these plants emit strong smells that will progressively decrease following the action of BSF larvae. For each test, a comparison was made with BSF larvae reared on PKM, and we calculated a comparative growth index (CGI, that is the growth in percent with respect to that obtained with PKM). It turned out that BSF larvae always grew at a slower rate than with PKM, but growth strongly varied between substrates (CGI from -5 to -77%), (Graph 7-1). It is worth noticing that the growth of BSF larvae was null when using water hyacinth as growth substrate. Moreover, larvae tried escaping from substrate in these circumstances; however, no significant mortality was observed.



Graph 7-1 **Growth of BSF larvae using different "ecosystem" plant substrates, in comparison to PKM. Values are the median growth rates measured in 3 replications. Percentages indicate the growth deficit in comparison to PKM**

These experiments indicate that BSF larvae can be reared on several vegetal "ecosystem substrates". The texture of plant residues varies between species and is very liquid for some of them (*Lemna spp., I. aquatica).* BSF larvae produced from ecosystemic plants have a greener colour, their body is less rigid (probably due to a lower dry matter content) and they are less active than those reared on PKM. The nutritional values of these larvae vary between growth substrates (Table 7-2), but their protein contents are systematically higher than in larvae produced from PKM, probably as a result of the higher protein contents of some of these substrates.

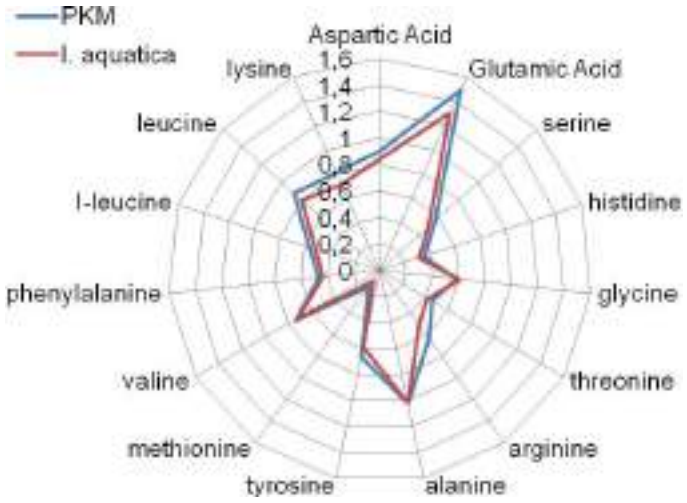
Table 7-1 Ecosystemic plants used as growth substrates for BSF larvae

Photo	Scientific Name	English name (Indonesian name)	Family	Information
	*Eichhornia crassipes*	Water hyacinth (Eceng gondok)	Pontederiaceae	Invasive plant in the tropical and subtropical zones (38°N to 38°S). Used as vegetable, fodder, green manure, compost, mulch or for water purification. Crude protein content: 12-20 % DM (whole plant) or 18 % (leaves).
	*Colocasia esculenta*	Taro (Talas)	Araceae	Cultivated in tropical and subtropical zones (Asia, Pacific Islands, Caribbean, Africa and South of USA). Edible corm and leaves. Crude protein content: variable but generally high (16-27% DM). Anti-nutritional factors: calcium oxalate (up to 3% DM – leaves).
	*Alocasia macrorrhizos*	Wild Taro (Sente)	Araceae	Native to southeast Asia. Edible tubers growing off the ground, ornamental variety. Broad leaves can achieve 1.5 m long, used to feed pig and fish (crude protein content: 24% DM).
	Genus Poaceae	Grass (Rumput)	Poaceae/ Gramineae	Garden grasses. Crude protein content: 12 % DM
	*Ipomoea aquatica*	Water spinach (Kangkung air)	Convolvulaceae	Invasive plant, sometimes cultivated in Southeast Asia (water purification water, edible young shoots). Can be used for feeding cattle, pig and fish. Crude protein content : 19% DM.
	*Leucaena leucocephala*	Leadtree (Pete)	Fabaceae	Fast growing tree, native to Central America and naturalized throughout the tropics. Edible leaves and seeds. Used as livestock fodder. High content in mimosine, a toxic acid for monogastric animals
	*Crotalaria spp.*	Cascavelle (Kacang Kacang)	Fabaceae	Seeds and pods can be toxic. Cultivated and used as fodder in India. Crude protein content: 15% DM (leaves).
	*Lemna spp*	Duckweed (Mata Lele)	Araceae	Small floating aquatic plant with fast growth, spreading at the surface of calm water bodies. Crude protein content: 7-20% DM in natural waters but up to 30-40% DM in waters rich in nutriments

Table 7-2 Proximate analyses of substrates (above) used for evaluating the performances of BSF larvae, and corresponding compositions of larvae (below)

Substrate	DM %	Protein % DM	Lipid % DM	Ash % DM	Fibre % DM
PKM	**92.0**	**16.2**	**12.4**	**4.4**	**22**
Lemna spp.	5.1	33.5	2.4	16.3	13.8
C. esculenta	5.2	29.5	7.1	14.1	34.2
Crotalaria spp.	20.8	24.0	2.9	6.3	23.0
A. macrorrhizos	6.5	21.3	5.5	14.6	38.6
I. aquatica	11.5	23.1	2.0	11.9	14.4
L. leucocephala	19.9	24.9	2.9	7.5	19.2
Poaceae grass	14.8	12.2	2.1	11.6	29.5
Larvae fed:	**DM %**	**Protein % DM**	**Lipid % DM**	**Ash % DM**	**Fibre % DM**
PKM	**28.0**	**38.8**	**37.6**	**11.4**	**14.9**
Lemna spp.	21.5	48.9	10.0	15.7	18.4
C. esculenta	21.9	50.1	10.0	15.8	22.5
Crotalaria spp.	28.5	48.2	7.8	17.2	19.8
A. macrorrhizos	23.7	48.5	3.1	22.2	28.3
I. aquatica	31.1	42.5	27.4	11.6	23.8
L. leucocephala	28.1	58.5	3.8	13.9	--
Poaceae grass	17.1	43.2	1.0	29.6	20.9

However this difference might also originate, at least in part, from the smaller body size of BSF larvae grown on some plants, as protein content is size-dependent. The amino acid profiles of BSF larvae produced with water spinach (*I. aquatica*) and PKM are similar (Graph 7-2). Lipid contents are always higher in larvae produced with PKM; however, the larvae produced with *I. aquatica* display a very high lipid content in comparison to larvae grown on other ecosystem plants.



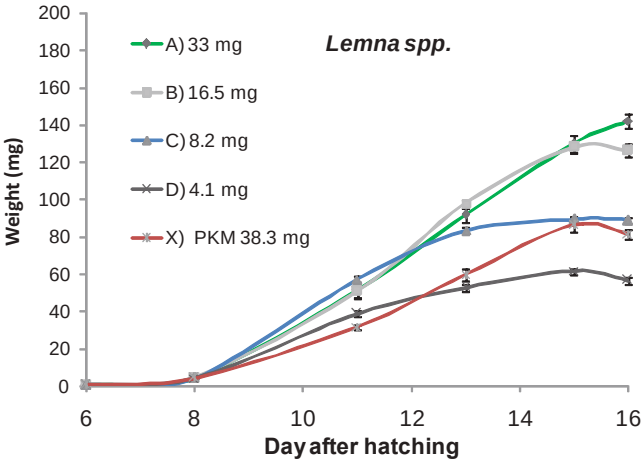
Graph 7-2 Amino acid profiles of BSF larvae grown on PKM and water spinach Ipomoea aquatica

Rationing and Growth of BSF using Ecosystem Plants

Dedicated experiments were performed to assess the effect of the daily food ration on the growth of BSF larvae using two promising ecosystem plants: water spinach *I. aquatica* and duckweed *Lemna spp*. Daily food rations were calculated on the basis of a geometric progression on a 2-log base, i.e. with every ration twice as high as the previous one. As for screening, BSF larvae grown on PKM in excess served as control groups. The amounts of substrates supplied during the first experiment (*I. aquatica vs.* PKM) were larger than in the second one (*Lemna spp. vs.* PKM*)*.

BSF larvae reared on both ecosystem substrates achieved faster growth than with PKM, even for low feeding levels. The difference was especially marked during the first feeding days. It is noteworthy that for any feeding level the growth of BSF larvae on plant substrates stopped abruptly between 14 and 15 dah (Graphs 7-3 and 7-4). The daily food rations that

are needed for producing BSF larvae with a body weight higher than 100 mg differ between the two plants. For *I. aquatica* at 57.5 mg per larva per day, FCR was 2.04 against 7.79 for PKM in slight excess.

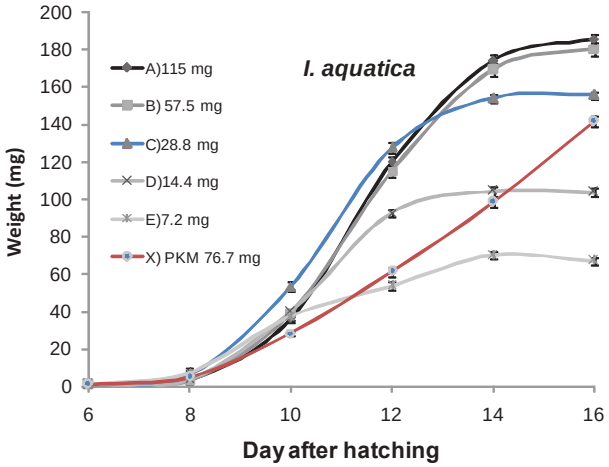


larvae mg/day DM	Day 6	Day 8	Day 11	Day 13	Day 15	Day 16
X) PKM 38.3 mg	ns	ns	ABC	ABC	ABD	ABD
A) 33 mg	ns	ns	XC	XD	XCD	XCD
B) 16.5 mg	ns	ns	X	XCD	XCD	XCD
C) 8.2 mg	ns	ns	XAD	XBD	AB	AB
D) 4.1 mg	ns	ns	C	ABC	XABC	XABC

Graph 7-3 Growth of BSF larvae using different feeding levels of water duckweed (Lemna spp.) and PKM. Symbols and whiskers refer to mean and SEM. The table below the graph indicates significant differences between feeding levels and substrates at α=0.05

The corresponding daily growth rates were 14 and 9.93 mg day^{-1}, with a 96% survival rate for *I. aquatica* against 81% for PKM. For *Lemna spp.,* using a DM rationing of 16.4 mg day^{-1} larva^{-1}, FCR was 1.24 against 5.42 for PKM. The corresponding daily growth rates were 12.6 against 8.6 mg day^{-1} and survival rates were 100% for both substrates.

Even if this particular study did not aim at comparing the performances of the two ecosystem substrates under evaluation (because of discrepancies between feeding levels), the contrast between the FCR obtained with these two plants is noteworthy. It clearly indicates that the growth of BSF larvae does not only depend on the amount of available substrate but also on its quality.



larvae mg/day DM	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16
X) PKM 76.7 mg	ns	ABE	ABCDE	ABCD	ABCE	ABDE
A) 115 mg	ns	XCE	XC	XDE	XDE	XCDE
B) 57.5 mg	ns	XCE	XC	XDE	XDE	XCDE
C) 28.8 mg	ns	ABD	XABDE	XDE	XDE	ABDE
D) 14.4 mg	ns	CE	XC	XABCE	ABCE	XABCE
E) 7.2 mg	ns	XABD	XC	ABCD	XABCD	XABCD

Graph 7-4 **Growth of BSF larvae using different feeding levels of water spinach (Ipomoea aquatica) and PKM. Symbols and whiskers refer to mean and SEM. The table below the graph indicates significant differences between feeding levels and substrates at α=0.05**

In these two experiments, the substantial differences between the FCR achieved with PKM and other plants have to be considered with care because PKM was supplied in excess. However, the faster growth and

lower FCR of larvae growing on ecosystem plants suggests their nutriments being more efficiently digested or assimilated. Because of its higher DM content, PKM has a higher nutriment content than ecosystem plants. For these two substrates, a fast liquefaction process was observed and this can account for a higher solubility and availability of nutriments.

These pioneer experiments with BSF larvae offer new opportunities concerning ecological intensification in rural aquacultural ecosystems. Other traditional agro-fish-farming systems may also benefit from BSF rearing and allow the transformation of many by-products, such as manure, rice-culture residues, orchard wastes, etc., the use of which is sometimes complicated or not straightforward in freshwater aquaculture. However this kind of BSF production, dedicated to small scale farms, depends on the availability of eggs or small larvae of wild BSF. Experiments of egg collection from wild BSF were performed in various areas of Guinea (Africa), Java and Sumatra.

They provided valuable insights on the efficiency of collection methods notably in forest areas, but they still need to be refined for securing this process. Thus, further studies have to be carried out especially with regard to both the economic feasibility of this technology in small farms and aspects pertaining to the collection of wild BSF eggs or young larvae.

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras

Chapter 8

Nutritional characteristics of *Hermetia illucens* for Fish Farming

Emilie Devic[(a)], Nina Meilizsa[(b)], Domenico Caruso [(a)], Pascale Talamond [(a)] and Yann Moreau [(a)]

(a) UMR 226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan

Several parameters govern the quality of fish food: it has to supply energy for physiological functions and growth, to be easily digestible, economically profitable, non-polluting and practical to use. The nutritional requirements of fishes can be highly specific, but fishes generally use proteins as the main source of energy for their vital functions. Fishmeal, which is rich in proteins, is extensively used in the formulation of fish food in aquaculture, but this resource is limited. Its replacement by other protein sources is a central issue for the sustainability of aquaculture, both for economic and ecological reasons. The use of plant proteins for industrial food production is systematic, but it is known that animal proteins display higher biological value than plant proteins. Indonesian fish farmers substitute frequently industrial food with hand-made food that incorporates significant amounts of "trash fish". This food is generally less performing and more polluting than fishmeal and it also has a significant ecological impact, as trash fish originate from coastal fisheries that heavily exploit demersal species and juveniles (Figure 8-1).

BSF, either as larvae or meal, can constitute an alternative to fishmeal or trash fish, at least if they have a suitable nutritional value and if they produce satisfactory survival, growth and conversion rates.

Figure 8-1 Small factory producing fish food in Sumatra. Left: feed already prepared from "trash fish". Right: trash fish drying before use

Nutritional Value of BSF Larvae

The composition of BSF larvae fed with PKM is given in Table 8-1. The dry matter (DM) content of BSF larvae grown on PKM in the pilot was 36.6%, of which about one third for their exoskeleton. Their crude protein content averaged 39.2% DM (Table 8-1), similar to the range (40-45%) for BSF larvae growing on other substrates (Hale, 1973; Sheppard *et al.,* 1994; Newton *et al.*, 2005a).

Table 8-1 Proximate composition of BSF larvae fed with PKM during three weeks. Values are percents of dry matter content. N=9, mean ± standard deviation. Analysis performed using AOAC.991.43 procedure (International AOAC 1998)

	Crude Protein (%)	Lipids (%)	Ash (%)	Crude fibre (%)
BSF larvae	39.2±4.1	38±4.9	9.8±1.6	19.5±4.9

However, there were substantial variations between the protein contents of BSF larvae in different experiments in the pilot (from 32 to 56%, based on 40 analyses). This is partly because BSF larvae were collected at slightly different ages, sizes or developmental stages, and the protein content is correlated with these factors. Variable food rationing during the experiments can also be responsible for these variations, because larvae fed *ad libitum* tend to accumulate larger amounts of lipids (and thus proportionally lower amounts of proteins) than those fed restricted rations.

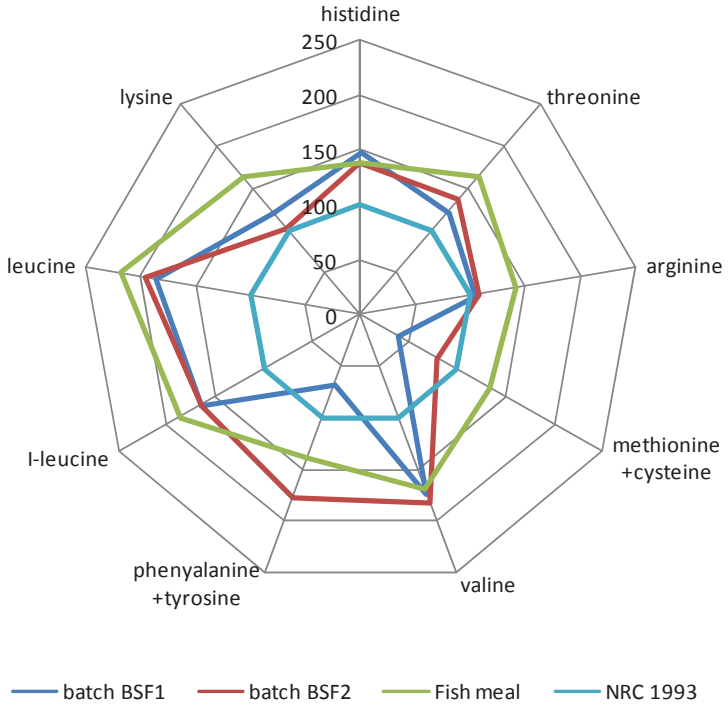
The determination of the protein content is performed using Kjeldahl method, which measures the total organic nitrogen content from both the BSF larva and the chitin (nitrogenous polysaccharide) coming from its exoskeleton. The chitin content of 3-week old BSF larvae grown on PKM is estimated at 4.5% of their dry matter (using fibre measurement with Van Soest method), consistent with the results of Finke, (2013); however Diener *et al*., (2009b) measured a higher chitin content (8.72% DM). The amino acid profile of a protein source and especially its content in essential amino acids (i.e. those that cannot be synthesised)

is an important parameter for estimating its dietary quality. The proteins of BSF larvae have a lower content in essential amino acids than in fishmeal, but their profile is well balanced and it meets the nutritional requirements of fishes (Graph. 8-1), except for sulphur amino acids (methionine and cysteine). Lysine and arginine are in the lower admitted range of concentration for fishes. The lipid content of BSF larvae grown on PKM was about 38%. This is slightly higher than reported in the literature (30-35%) with manure or other organic wastes. This might be due to the high lipid content of PKM. Indeed, the storage of lipids by BSF larvae follows a dose-dependent process, i.e. the higher the lipid content in the growth substrate, the higher the lipid content of animals growing on this substrate. Furthermore, lipid accumulation varies with the age and size of BSF: during larval development, their lipid content increases from about 13% to about 40% before pupation.

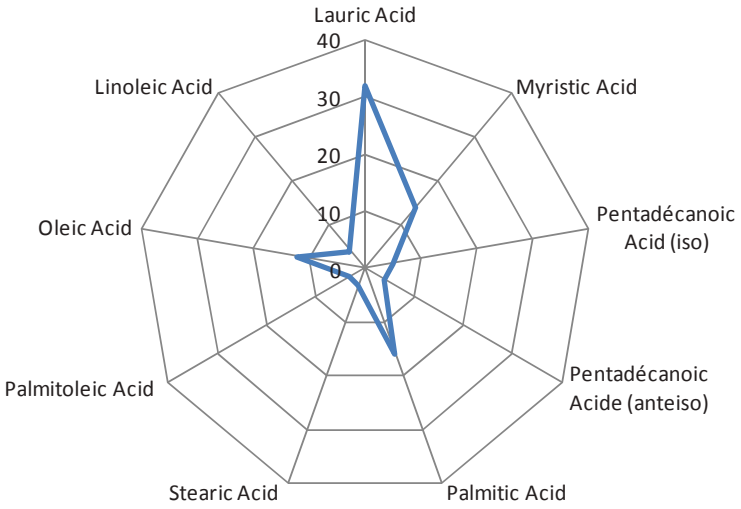
Estimates of the fibre content of BSF larvae vary substantially between the methods used for their analysis (Table 8-2).

Chitin is the main component of the cuticle (or exoskeleton) of BSF larvae. This nitrogenous polysaccharide is a long-chain polymer of N-acetylglucosamine (a derivative of glucose). Its structure compares to that of cellulose. When using conventional analytic methods, chitin cannot be discriminated from other fibrous material.

This can account, at least in part, for why the crude fibre content of BSF larvae was about three times as high here as in other data from the literature (20.7 % *vs.* 7%). This discrepancy might be due to the occurrence of undigested PKM, the overestimation of chitin or the presence of other polymeric protein structures.



Graph 8-1 **Essential amino acid profiles of 3-week old BSF larvae fed with PKM (two batches, BSF1 and BSF2) in comparison to fishmeal (Feedipedia, 2012). Values are expressed in % of crude protein contents and compared with the nutritional requirements of fishes (NRC, 1993 in Médale and Kaushik, 2009).**



Graph 8-2 Fatty acid profile (% of fatty acids) of 3-week old BSF grown on PKM

Table 8-2 Fibre content (% of dry matter) of 3-week old BSF larvae (following the methods by Hart and Fisher method, 1971)[2] Van Soest and Wine, (1967) ADF: Acid Detergent Fraction, and NDF: Neutral Detergent Fraction)

g/100g DM	Dry Matter (%)	Crude fibre[1]	NDF[2]	ADF[2]
BSF larvae 3-week old	86.8	20.7	13.5	11

The calcium content of BSF larvae generally varies from 2.4 to 5.0% DM (Finke, 2013; Newton *et al.,* 2005a), whereas larvae fed with PKM exhibited much lower contents (1.2% DM). This might be due to the growth substrate or to lower water hardness. Recently, Finke (2013) performed a complete study of the nutriment contents of BSF larvae and reported high contents of vitamins A, B, C, and D.

BSF for Fish Diet

BSF larvae can be used as fresh, frozen, freeze-dried or meals for animal feeding. However, very few studies have been dedicated to the use of BSF for fish feeding. The first experiments of BSF larvae as fish-food occurred in the 1980s, mainly on species such as the American catfish *Ictalurus punctatus,* the blue tilapia *Oreochromis aureus* and the rainbow trout *Oncorhynchus mykiss.* Bondari and Shepard, (1987) have shown that the substitution of 10% of fishmeal by BSF meal produces a slower growth of American catfish (after 15 rearing weeks). Likewise, the substitution of fishmeal by BSF meal results in a significant decrease of rainbow trout growth as soon as the substitution rate exceeds 25%. On the other hand, St Hilaire *et al.,* (2007) found that the consumption index increased significantly for rates of 50% substitution. Similar results were noted by Sealey *et al.,* (2011), who also reported that trash fish added in growth substrate (hog manure) partly reduced the negative effect of fishmeal substitution by BSF meal. As regards carps, the substitution of less than 20% of fishmeal by BSF larvae did not produce any significant variation of zootechnical performance (growth, survival, food efficiency; Cahyoko *et al.,* 2011). In Guinea, BSF larvae have been used for the experimental feeding of Nile tilapia *O. niloticus* in rural fish farms. Nile tilapia fed over six months with a diet made of 70% rice bran and 30% BSF larvae produced from PKM exhibited a daily growth rate three times as high as others exclusively fed with rice bran (1.80 *vs.* 0.52 g day^{-1}; Hem *et al.*, 2008).

BSF larvae have been recently evaluated as an alternative to fishmeal in the diet of juvenile turbot (*Scophthalmus maximus*). Their growth was satisfactory but nevertheless remained significantly lower than with fishmeal, and growth reduction was proportional to the rate of substitution of fishmeal with BSF meal. In particular, the food conversion rate (FCR) of juvenile turbot increased significantly for BSF meal inputs higher than 33%. It is likely that the lower performance of BSF meal (in terms of fish growth and conversion efficiency) is due to its lower digestibility. With a 30% BSF in fish food, visible digestibility coefficients decrease by 36.6, 22.1,

15.9 and 27.3% for organic matter, crude protein, lipids and raw energy, respectively, in comparison to a standard fish food made essentially of fishmeal (Kroeckel *et al.,* 2012).

Experiments on the partial or complete substitution of fishmeal by BSF larvae led to a collaborative research effort between French and Indonesian researchers (BPPKP-IRD). These experiments were performed on various fish species (especially catfishes belonging to the family Pangasiidae) that are central to fish farming in Southeast Asia. This research demonstrated that results varied between species. For example, the substitution of fishmeal by BSF larva meal produced no significant difference of SGR for *Pangasius djambal*, at least for substitution rates lower than 22%. Nevertheless, Ng *et al.,* (2002) found that the substitution of fishmeal by 22% PKM had no negative impact on the growth of other fish (*Oreochromis* spp.) fed this diet. Further experiments provided evidence that the growth of fish fed with both sources, i.e 24% PKM and 17% BSF larva meal was not decreased significantly in comparison to fishmeal. Henceforth, about 40% of fishmeal proteins can be substituted without any loss of fish growth when both PKM and BSF larvae are used altogether. This suggests a synergic or compensatory effect that remains to be investigated (Moreau, 2010).

The proportion of fishmeal proteins that can be substituted without penalty as regards growth or food conversion by fish larvae or juveniles varies between species. Concerning *Pangasianodon hypophthalmus*, it is possible to substitute 30% fishmeal by BSF meal without any SGR loss, but a linear decrease of SGR is observed for higher substitution rates. As a matter of fact, the substitution of as much as 81% of fishmeal proteins by BSF larva meal remains possible for *P. hypophthalmus*, but their rearing period is about 30% longer, and their FCR is slightly higher as well. Concerning hybrids of tilapias of genus *Oreochromis,* SGR decreases slowly for low substitution rates, then strongly for higher rates. In the latter case, the rearing period for attaining the desired fish size increases by at least 50% and the food supply requested to reach this goal increases by over 60%.

Some fish species can be fed exclusively with BSF larvae, but to the detriment of growth performances. Channel catfish (*I. punctatus*) fed 100% BSF larvae exhibit a slower growth and worse conversion index than when they are given alternative diets. Likewise, the feeding of *O. aureus* with 100% BSF larvae leads to a significantly slower growth in comparison to a complete diet. However, a significant improvement is noticed if BSF larvae are minced prior to distribution. According to Bondari and Sheppard, (1987), these growth rates are slower because of the low dry matter content of BSF larvae.

As regards the Indonesian catfish *Pangasius djambal*, no significant growth decrease was observed when commercial pellets were substituted with BSF larvae up to 25-30% (dry weight) in hapa or 40-45% in aquarium. With a replacement rate of 100%, the growth period was twice as long as with pellets, and the amount of food (DM content) increased by 62% (situation in hapa). With a substitution rate of 75%, additional food was not necessary, but growth was slower. These results offer alternative feeding strategies to fish farmers of *P. djambal* if fast growth or short rearing periods are not a premium (Moreau, 2010). Similar experiments on fingerlings (10-20 g) of *P. hypophthalmus* have shown that fish survival was not modified when BSF larvae were substituted to their standard diet, even at high rates. Nevertheless, it matters that the size of BSF larvae be compatible with the mouth opening and feeding behaviour of the fish.

Channa micropeltes is a piscivorous species that is usually fed on trash fish (not on pellets). In this particular species, the substitution of trash fish with BSF larvae did not affect survival and SGR were not significantly modified when part of the trash fish (up to 50%) was substituted by live BSF larvae. Reduction of weight gain and increase of FCR were observed at higher doses of substitution (Ediwarman *et al.,* 2008).

Some fish species are well adapted to this type of food. Devic and Hem (2011, unpublished data) obtained satisfactory growth rates (1.7-2 g per day and SGR of 1.1-1.2% per day) in 50-300 g giant gourami *(Osphronemus gouramy)* fed exclusively on BSF larvae (Figure 8-2).

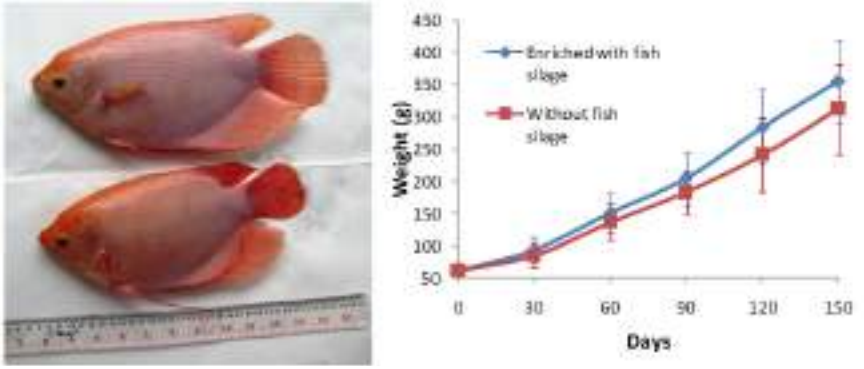


Figure 8-2 Osphronemus gouramy (Padang variety) reared in cement tanks and exclusively fed BSF larvae. Two types of BSF larvae were evaluated: larvae grown on PKM (red line) and others on PKM enriched with ensiled fish residues (blue line). The difference between growth rates is not significant

BSF larvae can thus be used as fish food, yet with a lower efficiency than fishmeal, but without any effect on their survival, at least as regards the species under study. It is likely that the slower growth of fish fed BSF larvae was partly due to the presence of chitin that most fish cannot digest. Moreover, chitin has been found to decrease the absorption and use of lipids by fish, or to be responsible for a reduction of their food intake and growth, even when present in low amounts in the diet (Shiau and Yu, 1999; Gopalakannan and Arul, 2006; Olsen *et al.,* 2006).

According to Kroeckel *et al.,* (2012), the low digestibility of BSF larvae could be due to their high content in saturated fatty acids. These authors indeed found that food digestibility in turbot decreased linearly with increasing proportions of saturated fatty acids in their diet. No similar effect was observed in the fish species under evaluation in Indonesia. In *P. hypophthalmus*, a food made of BSF larvae (genuinely rich in lipids) was compared with a food enriched with saturated lipids (from palm oil) and a lipid-poor food. Results showed that fish fed the BSF food grew at a slower rate than those given the food enriched with lipids, thereby

indicating that the negative effect of BSF larvae on fish growth did not originate from its high lipid content (Moreau, 2010).

Appetence of BSF larvae may vary according fish species. Youngs of *P. hypophthalmus* are reluctant to ingest BSF larvae or meal. In fingerlings (5-13.5 g) of this species, the substitution by BSF meal (≥ 10 %) leads to significantly lower food intake (Caruso, unpublished data). In terrestrial vertebrates, a satiating effect has been attributed to coconut oil, which is rich in lauric acid (C:12), as are the larvae of BSF. This satiating effect might be due to both fast oxidation and low incorporation of this medium-chain fatty acid. In the rainbow trout *(O. mykiss),* the significant addition of lauric acid in diet (57% of food intake) does not cause any decrease in food intake, but this species is capable of desaturating or elongating C:12 fatty acid (Figueiredo-Silva *et al.,* 2012).

In conclusion, BSF larvae represent a promising alternative to other sources of animal protein for fish feeding due to their high protein and energy contents and to their balanced amino acid profile. However, chitin, medium-chain saturated fatty acids and other compounds those remain to be studied in order to reduce their efficiency. These disadvantages could be alleviated by appropriate technologies. These include, for example the fibre content of BSF larvae grown on anaerobically degraded PKM that shows a decrease of 40% to 50% of the ADF fraction (which measures chitin, cellulose and lignin), or food enrichment with chitinolytic bacteria. Defatting and/or addition of palatable substances in BSF meal can also improve its appetence.

These solutions will not be considered until the production of BSF larvae meets the quantitative and qualitative needs of the animal feeding industry. Moreover, the nutritional value of BSF larvae strongly depends on their growth substrate. Nevertheless, BSF larvae can already represent a viable alternative to other sources of animal proteins in small fish farms that nowadays suffer from the irregular supply or high cost of fishmeal and formulated feed.

Feeding Trials on Other Animal Species

Larvae of *H. illucens* could be used for feeding many other species of monogastric animals. Insects are considered as a suitable new food source for poultry Oluokun, (2000) and Khusho *et al.,* (2012) showed that the substitution of 10 % of soybean meal by the same amount of BSF meal improves weight gain, conversion rate and carcass yield. These positive effects are similar to those obtained when adding 10 % of fishmeal. Elwert *et al.,* (2010) reached the same conclusion by using BSF meal for both starting and fattening food. These authors suggest that the degreasing of BSF meal is counterproductive. As regards pig farming, the apparent digestibility of BSF meal nutrients (fibre, ash, nitrogen, lipids, etc.) can be lower or higher to that of soybean meal, depending on contexts and reports. The appetence of foods containing BSF or soybean meal is similar (Newton *et al.,* 1977). BSF larvae are well adapted to the feeding of insectivorous birds, lizards or other reptiles in captivity, except for their energy, partly because their dry matter ratio, is too high (Finke, 2013). On the other hand, Bodri and Cole, (2007) do not recommend them for the feeding of carnivorous species as, for example, young American alligators (*Alligator mississippiensis*).

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras

Chapter 9

Economic Aspects of Production

Domenico Caruso [(a)], Lili Sholischah [(b)] and Emilie Devic [(a)]

(a) UMR226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) BPPBIH (Balai Penelitian dan Pengembangan Budidaya Ikan Hias) Jl. Perikanan No. 13, Pancoran Mas, 16432 Depok, Jawa Barat, Indonesia.

The BSF rearing unit at Depok was initially designed as a scientific tool for the study of BSF domestication and propagation in captivity, and not for the optimization or production costs or harmonization of the whole production process (pupae → breeders → eggs → mass production of larvae). It evolved into a mass production prototype, but which suffered from the imbalance between the dimensions of its productive components, the insectarium being oversized in comparison to the larvarium. The economical analyses developed in the present chapter are affected by this imbalance.

Calculations behind the annual breakdown come from several tests of mass production of fattening larvae in the larvarium at Depok. As regards the production of eggs and broodstock, the balance sheet was calculated on the basis of a monitoring of egg production over several months, which indicated an average weekly production of 394±282 g of eggs[1], i.e. an annual production of about 20 kg. In order to make calculations easier, this value has been converted into **'egg units' (EU) equivalent to 10 g of eggs**. Therefore, the pilot produces 2049 EU per year. Every week 4.4 EU are used for the renewal of BSF broodstock in the insectarium, thus 229 EU per year, to which one must add 11.4 EU per year incompressible to maintain a minimal stock of BSF. Indeed, even though the producer does not intend producing eggs for the subsequent production of fattening larvae, he nevertheless must maintain the biological cycle throughout, because starting an entirely new production from scratch can be difficult or very long. Finally, 1809 EU are available for the production of fattening larvae, thus about 35 EU per week.

On the basis of observed results under conditions of mass productions in the pilot at Depok, each 10-g EU can produce on average 23.8 kg of BSF larvae every 9-14 days. Therefore the annual production of BSF larvae in these conditions could amount to about 43 tons. Still by reference to the production results in the pilot, every EU uses a 4-m^2 production tank for about 2 weeks (9-14 days, depending on larval growth; cf. Chapters

1 These data differ from those given in chapter 4 (215 ±142 g), because other production data have been taken into account here

1 and 6). Hence, to accommodate all 35 EU produced every week in the insectarium, a total of 70 production tanks would be needed (overall production surface area of 280 m^2). With an average conversion rate of 2.18 observed in the pilot (cf. Chapter 6), about 100 tons of PKM (with residual moisture of 8 %) would be necessary every year to fuel the production of BSF fattening larvae. These values (70 production tanks, 100 tons of PKM per year) were used for producing the balance sheet for the "production of fattening larvae" so as to homogenise the production capacities of the larvarium and insectarium. Trading accounts here consider exclusively the economic results coming from the (fictive) sale of BSF larvae. Several by-products of the bioconversion process (fertilizer, chitin from exuviae) are also valuable (cf. Chapter 5) and can improve operating results (*cf.* "Promotion of by-products").

Promotion of By-Products

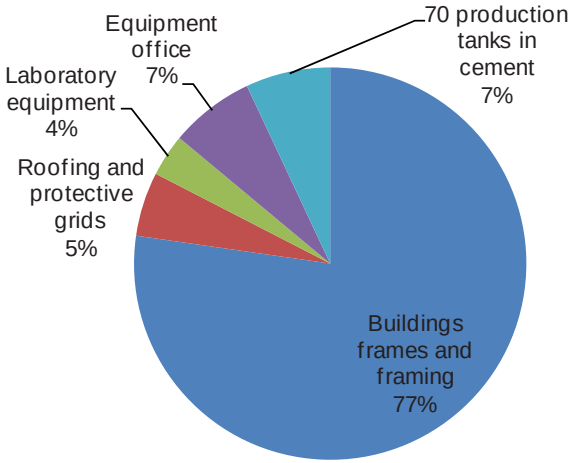
The market of organic fertilizers is booming in Indonesia and elsewhere in the world. The amounts of biofertilizer coming from the productions of BSF pupae for broodstock renewal and fattening larvae are significant. As suggested by the results of experimental productions (Cf. Chapter 6), these amounts could be as high as 8 tons per year for the former and 25-45 tons per year for the latter production, although with a slightly higher water content. It is difficult to estimate the value of this biofertilizer, as it depends on its intrinsic quality, but also on its origin, availability and market demand. However, in 2009, the Jakarta Post reported a price increase (from 7,000 to 10,000 Indonesia Rupiah, IDR; Benget, 2009), thus 0.59 - 0.85 **€**[2] per kg of biofertilizer. Therefore it could be a major source of additional incomes for BSF producers. However, the acceptance of this product remains to be evaluated. Another by-product of BSF production can generate significant incomes. Exuviae of insects are rich in chitin and calcium carbonate. Chitin is used for a broad range of purposes in various domains such as chemistry, pharmacy, phytosanitary and industrial products. Proportionally, exuviae of BSF are richer in calcium carbonate than in most other insects. On the basis of the calculations above, the production of BSF exuviae could amount to 3.2 tons per year in the pilot at Depok. The chitin level in BSF exuviae has not been determined yet. In the domestic fly *Musca*

2 Exchange rate: 1**€** =12013.73 IDR, August, 2012, <Www.xe.com>.

domestica, it is about 45 % (Kramer and Muthukrishnan, 2009). If this proportion applies to BSF, then 1.44 ton of chitin could be produced every year in the pilot. Selling prices vary considerably according to chitin purity and indications for use, from 4 to over 80 **€** per kilogram. It is thus important that future research examines the chitin contents of BSF exuviae, ways of extraction and potentialities of valorisation.

Investments and Depreciation

The breakdown of investments is shown in graph 9-1. Here, investments do not take into account any mechanization of the production. In other conditions, it could be useful or indispensable, notably for heavy and hard tasks. Nevertheless, the machinery that would be best for these purposes remains to be identified or designed, and its cost or cost-benefit ratio should be evaluated to determine the degree of priority for this particular investment.



Graph 9-1 **Breakdown of investments in order to create a production unit of BSF. This calculation takes into account the building of 70 productions tanks for matching the production capacities of the larvarium and insectarium (not realized in Depok)**

Here, building costs contribute to over 75% of the total investment. Furthermore, this value does not take into account the cost of land property, otherwise it would be higher. However, for most small scale farmers interested in the development of BSF rearing as a complementary activity, this should not be a major issue, as they already are land owners. Nevertheless, the interest of using a part of their land for the building of BSF rearing structures remains to be compared with alternative investment opportunities. The cost of land property in peri-urban or industrial areas is generally high and it can increase significantly the overall investment, especially because land acquisition generally does not apply for amortization

Detailed investment costs are given in Table 9-1. They are largely based on the building costs of the production pilot at Depok, but here with a larger larvarium to match the production of BSF eggs in the existing insectarium. This table is subdivided into two parts. The first one refers to the investments needed for the production of BSF broodstock and eggs (insectarium and associated puparium), and the second one to the larvarium for the production of BSF larvae. In this context, the total investments amount to 516.3 million IDR (44 132 **€**), of which the largest part (74%) is dedicated to the production of BSF broodstock and eggs.

The costs dedicated to the insectarium building amount to almost 59% of total investments, because of its more robust construction and sophisticated design. The adequate size of this particular structure should thus be thought out wisely by future producers of BSF. Various types of buildings initially used for other agricultural productions can also serve this purpose. However, especially for the insectarium, it is important that the building be well designed to prevent the entrance of predators and the escape of adult BSF.

In view of the preferences or requirements for BSF adults and eggs as regards temperature and light intensity, the construction should be made of transparent or translucent material (glass or plastic) and steel structures. The insectarium used for BSF egg production can thus differ to a large extent from the prototype built in Depok, but its building will nevertheless be expensive, because of high cost of technology or materials.

Experience has shown that the insectarium in the production pilot at Depok was largely oversized, and that its productivity could be much higher than observed here (i.e. egg production could be at least 5-10 times higher in view of the fecundity of female BSF; cf. Chapter 4). From an economic point of view, the total surface of the insectarium has to be dimensioned wisely, as building depreciation here accounts for 17.7% of the total production costs.

However, it is difficult to determine the adequate dimensions of the insectarium before any previous experience with the actual performance of all components of the production system. Alternatively a modular design can be considered for the insectarium.

The issue of rearing density, both for BSF larvae and adults, can be critical, as it governs production and needs for land property or building size. Very few experiments on the effect of rearing density were conducted in the production pilot at Depok, and their results do not enable drawing any reliable predictions as regards the upscale or downscale of production structures. Future BSF farmers will have to find out the best strategy for intensification, ideally following a stepwise process, in order to maximise returns on investment.

Operating Accounts

The operating accounts below describe an analysis of fixed and variable costs, and their breakdown into the two components of the BSF production system over one year (i.e. broodstock renewal and egg production on the one hand, and production of fattening larvae on the other hand). As indicated above, the operating accounts for the former component refer to productions already carried out in the production pilot at Depok, whereas for the latter component, they refer to an upscaling simulation. Tables 9-2 and 9-3 show the total annual production costs, the corresponding costs per egg production unit (10 g of eggs) and per kg of fresh BSF larvae, as well as their breakdown into fixed and variable costs. Some costs, such as laboratory and office equipment or wages, apply to both components of the production system. For the sake of simplicity, their breakdown follows a 4:1 ratio, on the basis of corresponding investments for buildings.

Table 9-1 Investments for the BSF production pilot in Depok

Investments and depreciation Egg production and breeders	Investments in IDR	Depreciation IDR per year	% of investments	% Total of investments
Buildings (frames and framing) 1100 m² at 285000 IDR (€ 24.32) per m² depreciation 25 years	313 500 000	12 540 000	82,43%	60,72%
Roofing and protective grids 1100 m² at 19500 IDR (1.66 €) per m² depreciation 8 years	21 450 000	2 681 250	5,64%	4,15%
Laboratory equipment depreciation 5 years	15 120 000	3 024 000	3,98%	2,93%
Equipment Computer, Printer, air conditioning, office furniture depreciation 5 years	30 240 000	6 048 000	7,95%	5,86%
INVESTMENTS and ANNUAL DEPRECIATION in IDR ***INVESTMENTS and ANNUAL DEPRECIATION in €***	**380 310 000** ***32 507,80 €***	**24 293 250** ***2 076,52 €***	**100%**	**73,66%**
Investments and depreciation production fattening larvae	**Investments in IDR**	**Depreciations IDR per year**	**% of investments**	**% Total of investments**
Buildings (frames and framing) 300 m² at 285000 IDR (€ 24.32) m² depreciation in 25 years	85 500 000	3 420 000	62,87%	16,56%
Roofing and protective grids 300 m² at 19500 IDR (1.66 €) per m² depreciation 8 years	5 850 000	731 250	4,30%	1,13%
70 production tanks (cement) pc 4x1 m 514000 IDR depreciation 15 years	36 000 000	2 400 000	26,47%	6,97%
Laboratory equipment depreciation 5 years	2 880 000	576 000	2,12%	0,56%
Equipment computer, printer, air conditioning, office furniture depreciation 5 years	5 760 000	1 152 000	4,24%	1,12%
INVESTMENTS and ANNUAL DEPRECIATION in IDR ***INVESTMENTS and ANNUAL DEPRECIATION in €***	**135 990 000** ***11 624,03 €***	**8 279 250** ***707,69 €***	**100%**	**26,34%**
INVESTMENTS and ANNUAL DEPRECIATION in IDR ***INVESTMENTS and ANNUAL DEPRECIATION in €***	**516 300 000** ***44 131,8 €***	**32 572 500** ***2 784 €***	**100%**	

The breakdown between fixed and variable costs for the production of BSF broodstock and eggs is given in Table 9-2. It appears that fixed costs are about 4 times as high as variable costs, as they require the permanent presence of 3 workers and many other expenses for the renewal of broodstock. As indicated above, these costs were calculated on the basis of the minimal number of adult BSF needed for producing 11.4 EU per year.

The variable costs of these operating accounts depend on the proportion of EU that will be used for the production of BSF adults or fattening larvae rearing (here, 229 and 1809 EU, respectively). They are exclusively linked to the purchase of PKM, its transport and handling (i.e. unloading), which are almost as expensive as PKM itself. PKM is widely produced in Indonesia, but its cost and availability are highly variable. In the present calculation, the cost of PKM is among the lowest prices paid in 2010 and 2011 at Depok (i.e. 558 IDR/kg, exclusive of transport and handling costs). However, this cost can be twice as high in other circumstances. Future BSF farmers will have to be very careful as regards the variability of PKM cost and availability over time. PKM storage over 1 year seemingly has no negative impact on its nutritional quality for rearing BSF larvae. Therefore, farmers can purchase larger amounts of PKM when it is abundant or cheap, but this requires sufficient cash flow and an additional investment for the building of storage structures.

It is also worth reminding that the consumption of PKM for producing BSF pupae is proportionately higher (by about 43%) than for the production of fattening larvae. Indeed, abundant feeding is essential for the accumulation of energy reserves at the larval stage in order to produce more abundant eggs and better egg quality at the adult stage, but this is to the expense of food conversion (3.76 *vs.* 2.18 for BSF fattening larvae). This value could be improved after future refinements of pupa production. Nevertheless, the improvement of FCR proportionally has a greater relevance for the production of BSF larvae, because variable costs (and especially the cost of growth substrate, thus PKM in the present case) amount to about 80% of their production costs (Table 9-3).

Table 9-2 Breakdown of production costs (fixed and variable) for BSF egg production

Spawning and egg production on annual bases	Total Fixed Costs	Fixed costs per unit of production (10 g eggs)	% of fixed costs	% Total production Cost
Fixed Costs	IDR	IDR		
Minimum broodstock (incompressible 11, 4 units producing eggs)	936 993	457	1,18%	0,96%
Consumable breeding broodstock (water + sugar + vitamin)	4 200 000	2 050	5,28%	4,28%
Actractant collecting eggs (fermented PKM)	332 010	162	0,42%	0,34%
Energy for Insectarium (electricity)	79 500	39	0,10%	0,08%
Other sources of energy Insectarium (Gas)	73 000	36	0,09%	0,07%
Energy for larvarium	0	0	0,00%	0,00%
Labor 3 UTH	41 600 000	20 303	52,32%	42,43%
Maintenance	2 400 000	1 171	3,02%	2,45%
Equipment security personnel (gloves, boots, safety shoes etc.).	1 600 000	781	2,01%	1,63%
Small material (plastic, bowls nets rake, shovel pipes)	4 000 000	1 952	5,03%	4,08%
Accumulated depreciation	24 293 250	11 856	30,55%	24,78%
Total Fixed Costs in IDR ***Total Fixed Costs in €***	**79 514 753** ***6 796,69 €***	**38 807** ***3,32 €***	**100,00%**	**80,15%**
Variable costs related to the production of eggs (1 production unit 10 g). Total units needed = 229	Total Variable costs	Variable costs per production unit (10 g eggs)	% of variable costs	% Total production costs
Supply (Water + PKM)	9 575 280	4 673	51,69%	9,77%
Transporting PKM	7 350 900	3 588	39,68%	7,50%
Additional labor (truck unloading PKM)	1 597 275	780	8,62%	1,63%
Total Variable costs in IDR ***Total Variable costs in €***	**18 523 455** ***1 583,33 €***	**9 040** ***0,77 €***	**100,00%**	**18,89%**
TOTAL COST FC + VC in IDR ***TOTAL COST FC + VC in €***	**98 038 208** ***8 380,02 €***	**47 847** ***4,09 €***	**100,00%**	

Table 9-3 Breakdown of productions costs (fixed and variable) for BSF fattening larvae

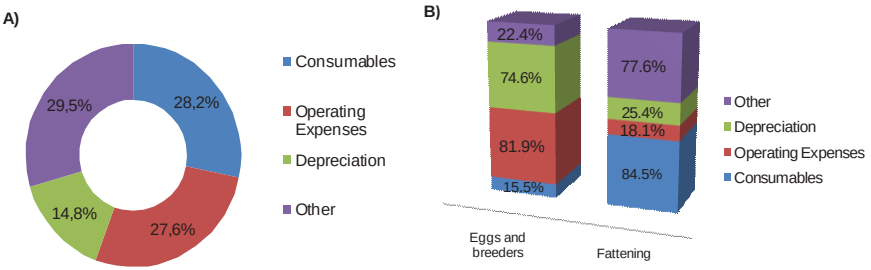
Production of fattening larvae on annual basis Fixed Costs	Annual costs	Cost per unit of production (1 kg of fresh larvae)	As % of fixed costs per unit of production (1 kg of fresh larvae)	% Total production Cost
Charges fixes	IDR	IDR		
Energy for larvarium	0	0	0,00%	0,00%
Labor 0.25 UT	10 400 000	242	50,29%	8,53%
Maintenance	600 000	14	2,90%	0,49%
Equipment security personnel (gloves, boots, safety shoes etc.).	400 000	9	1,93%	0,33%
Small material (plastic, bowls nets rake, shovel pipes)	1 000 000	23	4,84%	0,82%
Accumulated depreciation	8 279 250	192	40,04%	6,79%
TOTAL charges fixes en IDR	**20 679 250**	**480**	**100,00%**	**16,96%**
TOTAL charges fixes en €	**1 768**	**0,041**		
Variable costs for the production of fattening larvae from 1809 Eggs Unit = 31 651 kg of fresh larvae	Annual costs	Cost per unit of production (1 kg of fresh larvae)	As % of fixed costs per unit of production (1 kg of fresh larvae)	% Total production Cost
Supply (Water + PKM)	52 372 851	1 217	51,72%	42,95%
Transporting PKM	40 162 408	933	39,66%	32,94%
Additional labor (truck unloading PKM)	8 726 878	203	8,62%	7,16%
Total Variable costs in IDR	**101 262 138**	**2 352**	**100,00%**	**83,04%**
Total Variable costs in €	**8 656 €**	***0,201 €***		
TOTAL COST FC + VC in IDR	**121 941 388**	**2 833**	**100,00%**	
TOTAL COST FC + VC in €	***10 423 €***	***0,242 €***		

Breakdown of Production Costs

Graph 9-2 illustrates the breakdown of production costs for the two components of production system. Operating costs represent the main contribution, essentially because of manpower expenses (86%). Indeed, running the production unit necessitates the presence of 3 operators. This manpower can seem excessive at first sight, but it is necessary in absence of mechanization and because some tasks need being completed rapidly, which would be impossible with fewer operators. As regards the production of BSF eggs and broodstock, operating costs are slightly higher than depreciation costs (cumulating 84% for both of them). The remaining 16% are dedicated to electrical power supply, broodstock maintenance, purchase of sugar and vitamins (graph 9-2b). As regards the production of BSF fattening larvae, most expenses come from consumables and essentially from the purchase, transport and handling of PKM that is needed in large amounts. These costs also represent the bulk (62%) of the consumables for the production of BSF eggs and broodstock. The rest is dedicated to allocations for workers' safety and the purchase of small equipment (plastic containers, sieves, mosquito nets, etc.) that are quickly damaged and have to be replaced frequently.

Variable costs for the two components of the production system in Depok took into account PKM (purchase, transportation and handling) but not water, which came from existing boreholes and was therefore free of charge.

Nevertheless, water is strictly indispensable for the degradation of PKM substrate and maintenance of humidity inside the insectarium. Here, a total volume of about 200-230 m^3 would be needed every year, i.e. about 7 m^3 of water per ton of BSF fattening larvae.



Graph 9-2 Breakdown of production costs for total production (A) and for the two components of the production system (B)

Taking into account fixed and variable costs, the annual production costs would amount to 220 million IDR (**18 800 €**), i.e. **5,110 IDR (0.44 €)** per kg of BSF larvae. It is worth noting that the cost of BSF egg production represents 58% of the total production costs (and the amortization of the insectarium alone no less than 44.6% of this total cost). The excessively large size of the insectarium and its weak productivity are largely responsible for this rather high production cost.

Alternative Economic Scenario

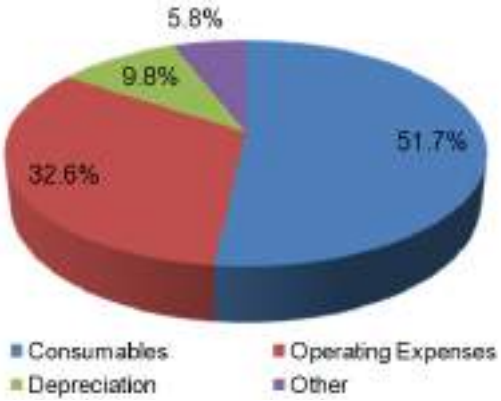
Under Depok pilot's conditions of production, it appears that the production of BSF larvae is not competitive with the price of fishmeal, whereas this ingredient is praised and considered as a rather expensive animal meal. Indeed, in order to produce 1 kg of BSF meal, about 4 kg of fresh BSF larvae are needed[3]. The production cost of BSF meal would thus be 17% higher than the selling price of fishmeal (currently at a free on board price of 1.41 **€** per kg for the Peruvian anchovy), and this estimate is optimistic, as it overlooks processing costs. Nevertheless, the selling prices of fishmeal increase regularly (+115% over the past ten years), so the balance could tip in favour of BSF in the near future, in Indonesia and elsewhere, with PKM or another growth substrate.

3 This is a restrictive hypothesis because the dry matter content of BSF larvae is about 1/3 of their fresh body weight.

Furthermore, the production costs of BSF larvae and meal can be lowered by: 1) reducing the size of the insectarium to 250 m^2 (provided that its productivity is higher than here), 2) reducing manpower (e.g. through better work organization or engineering for time-consuming tasks), 3) using lower (or null) amounts of sugar and vitamins for adult insects, as their positive effects have not been demonstrated, and 4) reducing the cost of PKM (including transport and handling).

This could be easily achieved if the BSF production unit was set nearby an oil mill, which produces large amounts of PKM as a by-product of palm oil processing.

In this context, the production cost of 1 kg of BSF larvae would drop to 2 782 IDR (0.24 **€**) instead of 5110 IDR (0.44 **€**) and BSF meal would then become about 32.7% cheaper than fishmeal. The breakdown of BSF production costs following this working assumption is illustrated in Graph 9-3.



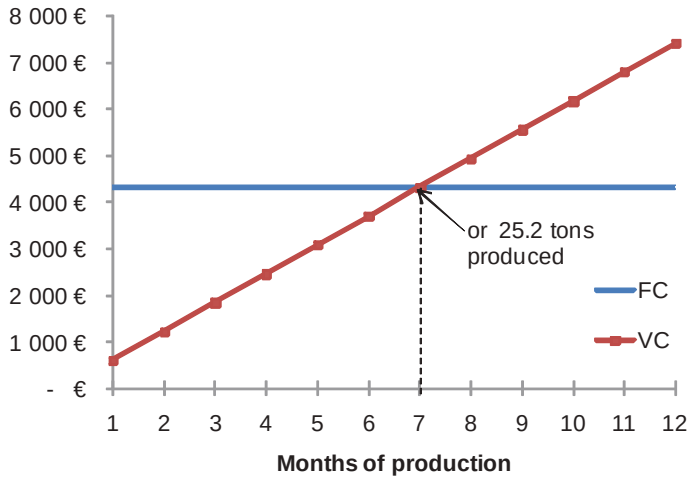
Graph 9-3 **Breakdown of total expenses for the production of BSF (larvae, eggs and broodstock), following the use of corrective actions (see text for details)**

The aforementioned production price of BSF must be incremented by the producer's profit margin, which depends on the prices of alternative resources for animal feeding (*cf.* Selling price, see below). The calculations below were made on the basis of a selling price of 3 616 IDR (0.31 **€** per kilo) per kg of BSF larvae and a gross profit margin of 30%. Table 9-4 shows the key figures for the production of BSF under this assumption, as in most financial analyses relying on the SIB method (Statement of Intermediate Balances).

Table 9-4 Statement of Intermediate Balances (SIB) for BSF production, as simulated from production results in the pilot at Depok, while using corrective actions concerning investments, fixed costs and variables costs. IDR: Indonesian rupiah

Stament of Intermediate Balance (SIB)			
Turnover	100%	155 662 432 IDR	13 306 €
Gross margin	60.2%	93 714 301 IDR	8 010 €
Added Value (AV)	55.7%	86 714 301 IDR	7 412 €
Gross Operating Profit (GOP)	30.68%	47 626 131 IDR	4 071 €
Operating Result (Excluding taxes and potential losses)	23.1%	35 922 100 IDR	3 071 €

As long as the entire production of BSF larvae is sold, this scenario produces an annual profit margin of 7% on the invested capital (516 million IDR). A more detailed analysis of this economic simulation is given in Figure 9-1. It appears that the break-even point can be attained by the 7th month of production or when the volume of produced BSF larvae amounts to 25.2 tons. These results are encouraging, but they can still be improved by proper management and further efforts as regard BSF rearing technology.



Calculations		
Fixed Costs (FC)	50 792 201 IDR	4 342 €
Selling price per 1 kg	3 616 IDR	0.31 €
Variable charges (per kg)(VC / 1 kg)	1 602 IDR	0.14 €
Contribution margin (CM)	2 015 IDR	0.17 €
Rate CM	56%	56%
Break-even point	91 178 012 IDR	7 794 €
Break-even point (quantity of larvae in kg)	25213	25213

Figure 9-1 Break-even point (BP) of BSF production in Indonesia, as estimated in a context where PKM cost is negligible (see text). FC: fixed costs; VC: variable costs

Zootechnical improvements can be expected to several respects. As regards the production of BSF adults and eggs, a better conversion rate of PKM can probably be achieved for the production of BSF pupae, and above all, egg productivity can be increased substantially to reduce the production costs of eggs. Likewise, a better conversion rate of PKM by BSF larvae would have a strong impact on their production costs.

The conversion rate of BSF larvae in experimental conditions was better than in mass production trials in the pilot, upon which calculations were based for the operating accounts above. These perspectives should enable some improvement of productivity in the near future. Nevertheless, the economic viability of BSF production will essentially depend on the management and optimization of PKM input (over 100 tons per year in our economic simulation). The improvement of zootechnical performance, a wise dimensioning of production and a strict management of inputs will be keys to the success of this production.

Selling Price of BSF

What could be the selling price for BSF larvae? Of course it depends on the market targeted by the producer. The selling price and profit margin will be higher for the food for exotic pets (e.g. lizard, ornamental fish, bird) than for livestock, but the volumes of sales will differ substantially as well. In the present handbook, the analysis focuses on the fish feeding market in Indonesia. As an echo to the aforementioned calculations, BSF meal can enter the market at a competitive price in comparison to fishmeal, especially in view of the variability in fishmeal prices, availability or accessibility to small farmers. In Indonesia, many fish farmers largely use trash fish or dried fish of local origin ("ikan rucah / kering"; 3 000-5 000 IDR / kg) as a source of animal protein for fish food. The production cost of BSF larvae is slightly below the above range, but this small difference may not suffice to ensure the widespread use of this new resource. Indeed, "ikan rucah" is rooted in Indonesian fish-farming practices. Therefore it is necessary to demonstrate that other advantages can be associated to the use of BSF larvae or meal by fish farmers (sanitary improvements, nutritional needs, ecological issues) or their production in comparison to alternative resources (e.g. recycling of by-products). The use of BSF larvae for fish feeding could be facilitated by specific conditions or opportunities, such as the development of waste treatment lines or social responsibility projects initiated by big companies (e.g. palm oil producers) in favour of small-scale farmers. In the latter case, the availability of the growth substrate for BSF larvae (PKM) is no longer a limiting factor for the development of their production.

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras
 ISBN: 978-979-493-610-8

Chapter 10

Conclusion and perspectives

Domenico Caruso [(a)] and Etienne Baras [(b)]

(a) UMR 226-IRD (Institut de Recherche pour le Développement) Graha Kapital 1, Jl. Kemang Raya No. 4, 12730 Jakarta, Indonesia.

(b) UMR 226-IRD-ISEM (Institut de Recherche pour le Développement-Institut Science de l'évolution Montpellier)

The Bioconversion project aimed at using the capacities of an insect, the black soldier fly (BSF) *Hermetia illucens*, to transform agro-industrial waste or by-products into a source of animal proteins and energy that can be used as an alternative to fishmeal by Indonesian farmers, and especially by fish farmers. This joint research project led by scientists from the Indonesian BPPKP and the French IRD involved the development of technologies for completing the life cycle of this species in captivity.

Following a series of dedicated experiments and production trials at a pilot scale, this research progressively led to the development of rearing technologies for the mass production of BSF larvae, which are described to an unprecedented extent in the present handbook. As with any innovation, some aspects of the production process still have to be improved and some experimental results need further validation before they can be upscaled at the level of mass production.

Nevertheless, current knowledge already suffice to raise the interest of a broad audience (scientists, fish farmers, decision-makers) for using the bioconversion process by BSF and associated technology as an alternative, and hopefully more sustainable way of producing, on the basis of local resources, sources of animal proteins that are indispensable for securing or enhancing aquacultural productions.

In view of their nutritional characteristics, fresh larvae of BSF or BSF meal can be a viable alternative to other protein sources for fish farming, while taking into account economical and ecological aspects. However, BSF larvae or meal are not a major contender of fishmeal (or other locally abundant sources of animal proteins) yet. Indeed, before BSF larvae or meal can enter the industrial formulation of fish food, producers will have to guarantee their nutritional quality, and thus to secure the continuous supply of high quality growth substrates.

Furthermore, the technology for processing BSF larvae into an animal meal that can be integrated into the formulation of industrial fish food will require additional studies. Nevertheless, BSF larvae can already be used as an alternative to fishmeal in small-scale food production units (for fish or other species) that are widespread throughout South-East Asia.

Indeed, BSF larvae are capable to transform nutrients from plants, residues and other agricultural by-products into compounds that are digestible by monogastric animals. They can thus be efficient in making the most of ecosystem resources that are abundant in traditional fish farms but poorly used until now. In other words, the use of BSF larvae can provide fish farmers with an affordable source of animal proteins for fish food, while giving them the opportunity to enhance the ecological potential of fish farm ecosystems, especially in remote areas where the availability and costs of inputs can be limiting.

On the whole, the development of insect production is a major issue for researchers and agro-industries that are looking for new food resources and alternative protein sources. Insects possess remarkable conversion abilities. Indeed, it is estimated that their food conversion efficiency for producing edible protein sources is 2 to 6 times as high as that for poultry, pig or cattle (Oonincx *et al.,* 2010). Furthermore, BSF larvae rearing produces a significantly lower amount of greenhouse gas emissions (GES) than livestock rearing and they might generate lower amounts of residues that can be detrimental to the environment.

The black soldier fly (BSF) has some major advantage over many other insects that could be used as alternative bioconverters. Unlike other insects such as *Tenebrio molitor* or *Acheta domesticus,* BSF larvae can cope with a broad range of environmental conditions (pH, temperature, humidity) and they can make the most of many growth substrates. Moreover, BSF is not a pest, so its rearing requires no specific precautionary measures, in contrast to other species such as *Musca domestica* or *Locusta migratoria*.

Furthermore, BSF rearing brings several collateral benefits, as for example reducing the smell of decaying organic matter or the production of biofertilizers. Its rearing also requires less manpower, because the accumulation of faeces of BSF larvae has no negative impact on their culture, largely because BSF larvae can recycle them to some extent.

Because of its many advantages, BSF can be a valuable candidate for mass rearing on agro-industrial wastes or by-products. As indicated by the

increasing number of research projects or applications around the world, BSF rearing has a strong potential.

In summary, the use of BSF larvae is currently:

- **Possible for small scale production units of animal food, but depending on rearing technology, and supply availability or cost (PKM or others);**
- **Possible and desirable for the ecological intensification of extensive fish culture, while making the most of ecosystem resources.**
- **Still premature for animal feeding industry (in absence of significant production and information on food processing).**

The production of BSF larvae has a low environmental impact. As a matter of fact, it can also serve the purposes of sustainable development to many respects. However, it is essential that fish farmers have no negative perception of BSF larvae or meal before this product can be used as an alternative to fishmeal or other protein sources. Moreover, there is currently no regulatory framework for the quality of this food source. To this respect, it will be indispensable to develop an appropriate regulation that will ensure the quality and traceability of BSF larvae productions.

The mass production of BSF larvae opens new fields of applications in addition to producing a new source of animal proteins for feeding fish or other monogastric animals. The list of “services” that have been or remain to be developed includes the treatment of liquid manure, other domestic and agro-industrial waste or the production of biodiesel. These perspectives, with an important economic and societal value, will still require further adaptations and research, but their interest is already supported by scientific results.

Projects of organic waste treatment by BSF larvae are of interest all around the world, as this insect nowadays has an almost cosmopolitan

distribution (except for coldest regions). For example, in the United States of America, the treatment of pig manure by BSF larvae has been successfully evaluated and implemented in different pilot structures, with a waste reduction by about 50% (Newton *et al*., 2005a). Hence, BSF larvae could largely contribute to reducing the pollution by nitrates in areas of intensive rearing. For developing countries or emergent countries, where sanitation structures are sometimes deficient, BSF larvae might also be an affordable and efficient local alternative. Such project is currently supported by a well-known foundation[1].

The industrial transformation of BSF larva and its by-products could also generate resources dedicated to other industrial activities for nutritional, cosmetic or pharmaceutical purposes. These activities could help consolidating the profits of BSF farming.

The domestication of BSF has just started, and data on the biology of this species are still fragmentary. Beyond its commercial applications, BSF also is a major field of interest for fundamental and applied research. We hope that this handbook will contribute to the development of the wise use of this insect to the benefit of mankind but with no negative ecological impact.

1 Bill and Melinda Gates Foundation

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras
 ISBN: 978-979-493-610-8

References

Adler A.I. & F.P.Brancato (1995). Human furuncular myiasis caused by *Hermetia illucens* (Diptera: Stratiomyidae). Journal of Medical Entomology, 32, 745-746.

Alimon A.R. (2004). The nutritive value of palm kernel cake for animal feed. Palm Oil Development 40, 12–14.

Anggraeni D. (2010). The effect of bioconversion fertilizer palm kernel meal (BFPKM) as fertilizer for the growth of *Vigna unguiculata* L. Walp (yard long bean) var. mutiara. [Thesis] for S2 graduation, University of Indonesia, March 2010, 95pp.

AOAC International (1998). Official Methods of Analysis of AOAC International. AOAC International, Gaithersburg, MD.

Ayuningtyas I. (2012). Biochemical and microbial changes during palm kernel meal (PKM) fermentation. [Thesis] for S2 graduation. Biotechnology, Institut Pertanian Bogor.

Belote J.M. & B.S. Baker. (1982). Sex determination in *Drosophila melanogaster*: Analysis of transformer-2, a sex-transforming locus. Proceedings of the National Academy of Science of the United States of America 79, 1568–1572

Benget B. (2009). Economic crisis helps boost growth in Indonesia's organic fertilizer sector. The Jakarta Post, Jakarta, March, the 2nd, 2009.

Berepubo N.A., Mepba H.D., Agboola O.J. & R.I. Onianwah. (1995). Inclusion rate and true metabolizable energy of palm Kernal Cake In broiler chicken diets in a humid tropical environment. Journal of Applied Animal Research 7, 27–34.

Boatright S.A. & J.K. Tomberlin. (2010). Effects of temperature and tissue type on the development of *Cochliomyia macellaria* (Diptera: Calliphoridae) Journal of Medical Entomology 47, 917-923.

Bodri M.S. & E.R. Cole. (2007). Black soldier fly (*Hermetia illucens*, Linnaeus) as feed for the American alligator (*Alligator mississippiensis*, Daudin). Georgia Journal of Science, 65, 82-88.

Bondari K. & D.C. Sheppard. (1981). Soldier fly larvae as feed in commercial fish production. Aquaculture 24, 103-109.

Bondari K. & D.C. Sheppard. (1987). Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque) and blue tilapia, *Oreochromis aureus* (Steindachner). Aquaculture and Fisheries Management, 18, 209-220.

Booth D.C. & C. Sheppard. (1984). Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): eggs, masses, timing, and site characteristics. Environmental Entomology, 13, 421423.

Bradley S.W., Booth D.C. & D.C. Sheppard. (1984). Parasitism of the black soldier fly by *Trichopria* sp. (Hymenoptera: Diapriidae) in poultry houses. Environmental Entomology 13, 451-454.

Bradley S.W. & D.C. Sheppard. (1984). House fly oviposition inhibition by larvae of *Hermetia illucens*, the Black soldier fly. Journal of Chemical Ecology 10, 853-859.

Cahyoko Y., Rezi D.G. & A.T. Mukti. (2011).The feeding effect of larvae meal (*Hermetia illucens*) in artificial feed on growth, feed efficiency and survival rate of common carp juvenile (*Cyprinus carpio* L.) Jurnal Ilmiah Perikananan Kelautan 3, 145-150.

Cerveró J.M., Skovgaard P.A., Felby C., Sørensen H.R. & H. Jørgensen. (2w010). Enzymatic hydrolysis and fermentation of palm kernel press cake for production of bioethanol. Enzyme and Microbial Technology 46, 177-184.

Comment, A. P., Initiative, N. U. A. F., & Sheppard, D. C. (2007). Black soldier fly prepuae: a compelling alternative to fish meal and fish oil. NOAA-USDA alternative Feeds initiative Report. 17pp.

Copello, A. (1926). Biologia de *Hermetia illucens* Latr. Revista de la Sociedad Entomologica Argentina 1, 23–27.

Desouhant E. (1997). Stratégies de ponte et traits d'histoire de vie chez les insectes. Exemple du balanin de la châtaigne, *Curculio elephas* (Coléoptère, Curculionidae), en conditions naturelles. PhD Thesis, Université Claude Bernard - Lyon I, Lyon, France, 173pp.

Diener S. (2010). Valorization of organic solid waste using the black soldier fly, *Hermetia illucens*, in low and middle-income countries. Swiss Federal Institute of Technology (ETH), Zurich, Switzerland.

Diener S., Solano N.M.S., Gutierrez F.R., Zurbrügg C. & K. Tockner (1983). Biological treatment of municipal organic waste using black soldier fly larvae. Waste and Biomass Valorization 2, 357-363.

Diener S., Gutierrez F.R., Zurbrügg C. & K. Tockner (2009a). Are larvae of the black soldier fly -*Hermetia illucens* - a financially viable option for organic waste management in Costa Rica? Proceeding of Twelfth International Waste Management and Landfill Symposium S. Margherita di Pula, Cagliari, Italy; 5 - 9 October 2009.

Diener S., Zurbrügg C. & K. Tockner (2009b). Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Management Research 27, 603-610.

Diener S., Zurbrügg C., Gutierrez F.R., Nguyen D.H., Morel A., Koottatep T.& K. Tockner (2011). Black soldier fly larvae for organic waste treatment - Prospects and constraints. Proceedings of the Waste Safe 2011 – 2nd International Conference on Solid Waste Management in the Developing Countries.

Düsterhöft, E.M., Posthumus, M.A., & A.G.J. Voragen (1992). Non-starch polysaccharides from sunflower (*Helianthus annuus*) meal and palm kernel (*Elaeis guineensis*) meal preparation of cell wall material and extraction of polysaccharide fractions. Journal of the Science of Food and Agriculture 59, 151-160.

Düsterhöft E.M., Bonte A.W. & A.G.J. Voragen (1993). Solubilization of non-starch polysaccharides from oil seed meals by polysaccharide-degrading enzymes. Journal of the Science of Food and Agriculture, 63, 211–220.

Elwert C., Knips I. & P. Katz (2010). A novel protein sources: Maggot meal from *Hermetia illucens*. Poster at the 11th Symposium on Pig and Poultry Nutrition, November 2010 Wittenberg, Germany.

Erickson M.C., Islam M., Sheppard C., Liao J. & M.P. Doyle (2004). Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Enteritidis in chicken manure by larvae of the black soldier fly. Journal of Food Protection 67, 685-690.

Everest Canary G. (2009). Diseño y gestion de un proceso para reciclar desechos organicos con la larva *Hermetia illucens* para producir harina de larva. Tesis de Maestria, Facultad de ingeniera Chia, Universidad de la Sabana, 106pp.

FAO (2002). FAO STAT Agriculture Data. Internet address : <http:/ /apps. fao.org>

Finke D.M. (2013). Complete nutrient content of four species of feeder insects. Zoo Biology 32, 27-36.

Feedipedia - Animal Feed Resources Information System - INRA CIRAD AFZ and FAO © 2012

Figueiredo-Silvia A.C., Kaushik S., Terrier F., Schrama J.W., Médale F. & I. Geurden (2012). Link between lipid metabolism and voluntary food intake in rainbow trout fed coconut oil rich in medium-chain TAG. British Journal of Nutrition 107, 17141725

Fuentes González O. & G. Risco Oliva (2009). First report of intestinal myiasis caused by *Hermetia illucens* (Diptera: Stratiomyidae). Revista Cubana de Medicina Tropical 61, 97-99.

Furman D.P., Young R.D. & P.E. Catts (1959). *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. Journal of Economic Entomology 52, 917-921.

Genç H. (2006). General principles of insect nutrition ecology. Trakya University Journal of Science 7, 53-57.

Géri C., Allais J.P., Beaudoin L. & F. Goussard (1995). Influence of consanguinity on the sex ratio and diapause of *Diprion pini* L. (Hym., Diprionidae) populations. II. Effect of inbreeding on diapause. Journal of Applied Entomology 119, 35-43.

Gopalakannan A. & V. Arul (2006). Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Aquaculture 255, 179–187.

Hale O.M. (1973). Dried *Hermetia illucens* (Diptera:Stratiomyidae) as a feed additive for poultry. Journal of the Georgia Entomological Society 8, 16-20.

Hamm R.L. & J.G. Scott (2009). A high frequency of male determining factors in male *Musca domestica* (Diptera: Muscidae) from Ipswich, Australia. Journal of Medical Entomology 46, 169-172.

Hart F.L. & H.J. Fisher (1971). Modern Food Analysis. Springer, Berlin, Germany.

Hem S., Fahmi M.R., Chumaidi, Maskur, Hadadi A., Supriyadi, Ediwarman, Larue M. & L. Pouyaud (2007). Valorization of palm kernel meal (PKM), a by-product from palm oil agro industry, via bioconversion: a natural process of particular interest for the development of aquaculture in Indonesia. Poster in International Conference on Oil Palm and Environment (ICOPE 2007), 15–16 November 2007, Bali, Indonesia.

Hem S., Toure S., Sagbla C. & M. Legendre (2008). Bioconversion of palm kernel meal for aquaculture: Experiences from the forest region (Republic of Guinea). African Journal of Biotechnology 7, 1192–1198.

Hem S. & Fahmi M.R. (2009). Production of live insect “mini-larvae” and use thereof for feeding aquarium fish, alevins of farm fish and pets. Patent WO 2009/136057.

Hogsette J.A. (1992). New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. Journal of Economic Entomology 85, 2291- 2294.

Hwang Y-S., Mulla M.S. & H. Axelrod (1976). Attractants for synanthropic flies. Identification of attractants and coattractants for *Hippelates* eye gnats (Diptera, Chloropidae). Journal of Agricultural and Food Chemistry 24, 164–169.

James M.T. (1935). The genus *Hermetia* in the United States (Diptera: Stratiomyidae). Bulletin of the Brooklyn Entomological Society 30, 165170.

Jeon H., Park S., Choi J., Jeong G., Lee S-B., Choi Y. & S-J. Lee (2011). The Intestinal bacterial community in the food waste-reducing larvae of *Hermetia illucens*. Current Microbiology 62, 1390-1399.

Jørgensen H., Sanadi A., Felby C., Lange N., Fischer M. & S. Ernst (2010). Production of ethanol and feed by high dry matter hydrolysis and fermentation of palm kernel press cake. Applied Biochemistry and Biotechnology 161, 318-332.

Kemppineen C.S. (1998). An investigation of some factors influencing oviposition and larvae development in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). Master's Thesis, University of Georgia, 132pp.

Khusro M., Andrew N.R. & A. Nicholas (2012). Insects as poultry feed: a scoping study for poultry production systems in Australia. World's Poultry Science Journal 68, 435-446.

Kim J.G., Choi Y.C., Choi J.Y., Kim W.T., Jeong G.S., Park K.H. & S.J. Hwang (2008). Ecology of the black soldier fly, *Hermetia illucens* (Diptera; Stratiomyidae) in Korea. Korean Journal of Applied Entomology 47, 337–343.

Kim W., Bae S., Park K., Lee S., Choi Y., Han S. & Y. Koh (2011). Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera:Stratiomyidae). Journal of Asia-Pacific Entomology, 14, 11-14.

Knudsen K.E.B. (1997). Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology 67, 319–338.

Kramer K.J. & S. Muthukrishnan (2009). Chitin metabolism in insects. pp. 497-530 In : Insect Development: Morphogenesis, Molting and Metamorphosis. (Gilbert L.I., ed.), Academic Press, San Diego, CA.

Kroeckel S., Harjes A-G.E., Knip I., Katz H., Wuertz S., Susenbeth A. & C. Schulz (2012). When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute - growth performance and chitin degradation in juvenile turbot (*Psetta maxima*), Aquaculture 364-365, 345-352.

Lardé G. (1990). Recycling of coffee pulp by *Hermetia illucens* (Diptera: Stratiomyidae) larva. Biological Wastes 33, 307–310.

Leclercq M. (1997). A propos de *Hermetia illucens* (Linnaeus, 1758) ("soldier fly") (Diptera, Stratiomyidae: Hermetiinae). Bulletin et Annales de la Société Royale Belge d'Entomologie 133, 275-282.

Lee H.L., Chandrawathani P., Wong W.Y., Tharam S. & W.Y. Lim (1995). A case of human enteric myiasis due to larvae of *Hermetia illucens* (Family: Stratiomyiadae): first report in Malaysia. Malaysian Journal of Pathology 17, 109-111.

Li Q., Zheng L., Cai H., Garza E., Yu Z. & S. Zhou. (2011). From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. Fuel 90, 1545–1548.

Médale F. & S. Kaushik (2009). Les sources protéiques dans les aliments pour les poissons d'élevage. Cahiers Agricultures 18(23), 103111.

Maddison D.R. & K-S. Schulz (eds.) (2007). The Tree of Life Web Project. Internet address : < http://tolweb.org >

Martinez M. (1986). Les diptères stratiomyidae (1ère partie). Cahier Liaison O.P.I.E. 20, 62.

Moreau Y. (2010). Nutrition and feeding of fish of aquaculture-interest in Indonesia. Final report. 2003-2009, IRD, Montpellier, France, 31 p.

Muangkeow N. & C. Chinajariyawong (2009). Determination of true amino acid digestibility and metabolizable energy in fermented palm kernel meal with Aspergillus wentii TISTR 3075 for chickens. Walailak Journal of Science and Technology 6, 231-232.

Mustafa M.F., Alimon A.R., Wan Zahari M.W., Ismail I. & M. Hair-Bejo (2004). Nutrient digestibility of palm kernel cake for Muscovy ducks. Asian-Australasian Journal of Animal Sciences 17, 514-517.

Myers H.M., Tomberlin J.K., Lambert B.D. & D. Kattes (2008). Development of black soldier fly (Diptera:Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37, 11-15.

Newby R. (1997). Use of soldier fly larvae in organic waste management. Proceedings of the "Compost 97" Conference, 14-15 July, Griffith University, Brisbane, Australia.

Newton G.L., Booram C.V., Barker R.W. & O.M. Hale (1977). Dried *Hermetia illucens* larvae meal as a supplement for swine. Journal of Animal Science 44, 395-400.

Newton G.L., Sheppard D.C., Watson D.W., Burtle G. & R. Dove (2005a). Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC, 17pp

Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC, 17.

Newton G.L., Sheppard D.C., Watson D.W., Burtle G.J., Dove C.R., Tomberlin J.K. & E.E. Thelen (2005b). The Black Soldier Fly, *Hermetia illucens*, as Manure Management / Resource recovery tool. Symposium on the State of the Science of Animal Manure and Waste Management, January 5-7, 2005, San Antonio, Texas, USA.

Ng W.K. & K.K. Chong (2002). The nutritive value of palm kernel meal and the effects of enzyme supplementation in practical diet for red hybrid tilapia (*Oreochromis* sp.). Asian Fisheries Science 15, 167-176.

Ng W.K., Lim H.A., Lim S.L. & C.O. Ibrahim (2002), Nutritive value of palm kernel meal pretreated with enzyme or fermented with *Trichoderma koningii* (Oudemans) as a dietary ingredient for red hybrid tilapia (*Oreochromis* sp.). Aquaculture Research 33, 1199-1207.

Ng W.K. (2004). Researching the use of palm kernel cake in aquaculture feeds. Palm Oil Developments 41, 19-21.

Oliver P.A. (2004). Disposal apparatus and method for efficiently bio-converting putrescent wastes. US 6780637.

Olsen R., Suontama J., Langmyhr E., Mundheim H., Ringo E., Melle E., Malde M. & G. Hemre (2006). The replacement of fish meal with Antarctic krill, *Euphausia superba* in diets for Atlantic salmon, *Salmo salar*. Aquaculture Nutrition 12, 280–290.

Oluokun J.A. (2000). Upgrading the nutritive value of full-fat soyabeans meal for broiler production with either fishmeal or black soldier fly larvae meal (*Hermetia illucens*). Nigerian Journal of animal science, 3(2).

Oonincx D.G.A.B., van Itterbeeck J., Heetkamp M.J.W., van den Brand H., van Loon J.J.A., & A. van Huis (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. Entomophagy and Environment 5(12), 1-7.

Pangestu D. (2009). Isolasi, identifikasi, dinamika, skrining pertumbuhan fungi dan fermentasi Palm Kernel Meal oleh fungi indigenos terpilih. [Thesis] for S2 graduation. Universitas Indonesia, Fakultas matematika dan ilmu pengetahuan alam, Biologi, Depok, Indonesia.

Poku K. (2002). Small-scale palm oil processing in Africa. FAO Agricultural Service Bulletin 148: 10 hlm.

Rachmawati (2010). Life history of *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae) on decaying Palm Kernel Meal. Thesis Sekolah Pascasarjana, Institut Pertanian Bogor, Bogor, Indonesia, 83 pp.

Robacker D.C., Moreno D.S. & A.B. Demilo (1996). Attractiveness to Mexican fruit flies of combinations of acetic acid with ammonium/amino attractants with emphasis on effects of hunger. Journal of Chemical Ecology 22, 499-511.

Saccone G., Pane A. & L.C. Polito (2002). Sex determination in flies, fruit flies and butterflies. Genetica 116, 15-23.

Sanchez L. (2010). Sciara as an experimental model for studies on the evolutionary relationships between the zygotic, maternal and environmental primary signals for sexual development. Journal of Genetics 89, 325-331

Schremmer F. (1986). Die polymetabole larval-entwicklung der waffenfliegenart *Hermetia illucens*. in Beitrag zur Metamorphose der Stratiomyidae. Annalen des Naturhistorischen Museums in Wien, Serie B, 88/89, 405–429.

Schütt C. & R. Nöthiger (2000). Structure, function and evolution of sex-determining systems in Dipteran insects. Development 127, 667-677.

Sealey W.M., Gibson T.G., Barrows F., Tomberlin J.K., Ross C. & S. St. Hilaire (2011). Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. Journal of the World Aquaculture Society 42, 34-45.

Sheppard, D. (1983). House fly and lesser house fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environmental Entomology 12, 1439–1442.

Sheppard D.C. (1992). Large-scale Feed Production from Animal Manures with a Non-Pest Native Fly. The Food Insects Newsletter 5(2), 3-4.

Sheppard C.D., Newton L., Thompson S.A. & S. Savage. (1994). A value added manure management system using the black soldier fly. Bioresource Technology 50, 275-279.

Sheppard C., Tomberlin J.K., Joyce J.A., Kiser B.C. & S.M. Sumner (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae), Journal of Medical Entomology 39, 695-698.

Shiau S.Y. & Y.P. Yu (1999). Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Orechromis niloticus* x *O. aureus*. Aquaculture 179, 439–446.

St-Hilaire S., Cranfill K., McGuire M.A., Mosley E.E., Tomberlin J.K., Newton L., Sealey W., Sheppard C. & S. Irving (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. Journal of the World Aquaculture Society 38, 309-313.

Sundu B. & J. Dingle (2003). Use of enzymes to improve the nutritional value of palm kernel meal and copra meal. Proceedings of Queensland Poultry Science Symposium 11(14), 1–15.

Sundu B., Kumar A. & J. Dingle (2008). Amino acid digestibilities of palm kernel in poultry. Journal of the Indonesian Tropical Animal Agriculture 3(2), 139–144.

Tacon A.G.J. & M. Metian (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285, 146-158.

Talamond P., Meryandini A., Ayuningtyas I., Fahmi M.R., Wayan S., Yamin M. & S. Hem (2011). Valorization of PKM (Palm Kernel Meal) by bioconversion. Poster in International Conference on Oil Palm and Environment ICO-PE 2011, 11–13 May 2011, Jakarta, Indonesia.

Tomberlin J.K. (2001). Biological, behavioral, and toxicological studies on the black soldier fly (Diptera: Stratiomyidae). PhD dissertation, University of Georgia, Athens. 101 pp.

Tomberlin J.K. & D.C. Sheppard (2001). Lekking behavior of the Black Soldier Fly (Diptera: Stratiomyidae). Florida Entomologist 84, 729-730.

Tomberlin J.K. & D.C. Sheppard (2002). Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. Journal of Entomological Science 37, 345-352.

Tomberlin J.K., Sheppard D.C. & J.A. Joyce (2002). Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95, 379-386.

Tomberlin J.K., Adler P.H. & H.M. Myers (2009). Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. Environmental Entomology 38, 930-934.

Van Soest P.J. & R.H. Wine (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the Association of Official Analytical Chemists 50, 50-55.

Vilarino M., Rudeaux F.,Leon A. & M. Picard (1996). Teneur en énergie métabolisable et digestibilité des acides aminés chez le coq de deux sous-produits de l'extraction de l'huile : tourteau de palmiste pressé (*Elaeis guineensis*) et germé et son de maïs extrait (*Zea mays*). Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux 49(3), 229-234.

Wong Y., Saw H., Janaun J., Krishnaiah K. & Prabhakar (2011). Solid-state fermentation of palm kernel cake with *Aspergillus flavus* in laterally aerated moving bed bioreactor. Applied Biochemistry and Biotechnology 164, 170–182.

Woodley N.E. (1989). Phylogeny and classification of the 'Orthorraphous' Brachycera. pp. 1371-1395 In: Manual of Nearctic Diptera, Vol. 3 (McAlpine J.F., Ed.), Entomological Society of Canada, Ottawa, Canada.

Yang C.H., Belawat P., Hafen E., Jan L.Y. & Y-N. Jan (2008). Drosophila egg-laying site selection as a system to study simple decision-making processes. Science 319, 1679-1683.

Yu G., Cheng P., Chen Y., Li Y., Yang Z., Chen Y. & J.K. Tomberlin (2011). Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. Environmental Entomology 40, 30-35.

Zhang J., Huang L., He J., Tomberlin J.K., Li J., Lei C., Sun M., Liu Z. & Z. Yu (2010). An artificial light source Influences mating and oviposition of black soldier flies, *Hermetia illucens*. Journal of Insect Science 10, 1-7.

Technical handbook of domestication and production of diptera
Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae.
Editors: Domenico Caruso, Emilie Devic, I Wayan Subamia, Pascale Talamond and Etienne Baras
 ISBN: 078-979-493-610-8

Glossary

Aedeagus: (plural aedeagi) is a reproductive organ of male insects.

Aerobic: in the presence of oxygen (or air).

Aflatoxin: mycotoxin (toxin produced by some species of fungi mainly *Aspergillus* spp.). These toxins are among the most carcinogenic substances known.

Allele: is a variant of a gene. Some genes have a variety of different forms, which are located at the same position, or genetic locus, on a chromosome. Alleles contribute to the outward appearance of the organism.

Anaerobic: in the absence of free oxygen or without oxygen.

Autosomal gene: a gene located on an autosome.

Autosome: are any chromosomes other than sex chromosomes (X-Y or Z-W, depending on species).

Chitin: one of the main components of the exoskeleton of insects and other arthropods (crustaceans, arachnids, etc.) and the most frequent polysaccharide besides cellulose on hearth.

Cohort: generational group that has experienced the same life events in a given period; in this technical handbook, a cohort indicates individuals born between two successive harvests of pupae.

Conspecific: which belongs to the same species.

Digester: an apparatus in which substances are softened or disintegrated by moisture, heat, chemical action, or the like. In this handbook, digester is the containers used for pupae and larvae production.

Discal cell: a large cell on an insect's wing located near the centre of the wing. This is a specific character of wings of some insects, including dipterans.

Ecosystemic resources: animal or plant resources supplied by an ecosystem, which give benefits to human beings. Also called ecosystem services, they are classified into four categories by the

Millenium Assessment (supporting, provisioning, regulating, and cultural). Their development is a key for ecological intensification in agriculture.

Emergence: hatching of the adult fly from its protective shelter (rigid cuticle), which is formed during the metamorphosis of the maggot into an adult fly.

Enzyme: complex protein produced by living cells and that catalyzes a chemical reaction

Epicarp: the most external layer of a fruit.

Exuviation: molting of arthropods, or rejection of the old carapace to allow growth. Concerning holometabolous insects (see chapter 5), exuviation is the exit from the pupa.

Exuvia (plural exuviae): cuticle or skin that is left after arthropods molting.

Fattening larvae: this is the final step of the grow-out of BSF larvae that are used for animal feeding.

FCR (Food Conversion Rate): zootechnical indicator of the animal efficiency to transform food into body weight. Food conversion efficiency decreases when FCR increases.

Fitness: property to survive and reproduction frequency of an organism or population. The fitness takes into account the number of gene copies that are transmitted to the next generation, the number of individuals per laying, etc.

Flagellomere: subsection of the flagellum, last segment of antennae in arthropods.

Halteres: pair of short projections in dipterous insects that are modified hind wings, used for maintaining equilibrium during flight, also called balancer.

Heterochromosomes or allosomes: sex-chromosomes (X-Y or Z-W, depending on species).

Hexose: monosaccharide (simple sugar) with 6 carbon atoms, such as glucose and fructose.

Holometabolous: also called complete metamorphism, is a term applied to some groups of insects to describe a specific kind of development that includes four life stages; an embryo or egg, a larva and an imago or adult.

Imago (plural imagines): final developmental stage at which the animal attains maturity. It is characterized by the presence of genital organs and wings in insects. This terminology is also used in Amphibians.

Insectarium: Building dedicated to maintaining live insect. Here is the building used for emergence, imago rearing, and egg production.

Larvarium: is the building used for grow-out of larvae and production of pupae.

Monogastric: describes animals such as pigs, poultry with simple single-chambered stomach in comparison to ruminants (cow, goat, sheep) that have a four-chambered complex stomach.

Muscidae: a family of flies found belonging to the super family Muscoidea; some of which are commonly known as house flies.

Mycetae: group of multicellular organisms of the fungi type, which collect their nutriments through the decomposition of organic matter.

Myiasis: Parasitic infestation of the body of a live mammal by fly larvae (maggots) that grow inside the host while feeding on its tissues.

Oviduct: passageway from the ovaries to the outside of the body. The ova (ovulated oocyte) travel along the oviduct to be fertilized by sperm.

Oviposition: the act of laying or depositing eggs.

Endophage-parasitoid: an insect larva developing within and feeding on the internal organs and tissues of the host, and that systematically leads to the death of the host when its own development is complete.

pH: measurement of hydrogen ion (solvated) activity. Solutions with pH lower than 7 are acidic whereas those with pH higher than 7 are alkaline.

Polymer: macromolecule made of repeated subunits, known as monomers (elastics and polystyrene are polymers).

Polyphagy: ability of an animal to eat various diets.

Prepupa: an inactive stage just before the metamorphosis (pupa) in the development of certain insects, including BSF.

Pupa (pupation): transformation stage of holometabolous insects that undergo a complete metamorphosis between the larva and the imago. Equivalent to chrysalis (or nymph) in lepidopteres (butterflies).

Puparium: wooden structure placed in the insectarium dedicated to the storage of pupae until the emergence of the imago stage.

Saprophagous: describes an organism feeding on decayed organic matter.

Sclerotisation: cuticle or exosketeton of arthropods becoming rigid

Scutellum: a term used in insect anatomy, is a posterior portion at the back of the 2nd segment of the thorax, located at the junction of a pair of wings.

Sex ratio: proportion of males and females in a given population of a species with sexual reproduction.

SGR: (Specific Growth Rate), is an indicator used to estimate the variation of the body weight during a certain period (in this handbook, SGR is the daily growth rate).

Tarsus: the most distal part of the leg of certain arthropods.

Volatile substance: substance that evaporates at ambient temperature.