Project FISH-DIVA

Maggot – Bioconversion Research Program in Indonesia Concept of New Food Resources Results and Applications 2005 - 2011

> Final Report Saurin Hem

November 2011

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Centre for Aquaculture Research and Developmen Research Institute for Ornamental Fish



Institut de recherche pour le développément

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SUMMARY

The bioconversion of Palm Kernel Meal (PKM) is a natural process consisting of extracting nutrients residue in this by-product, into a form of insect larvae biomass. This is a new source of animal proteins and fats for aquaculture. The insect in the bioconversion process (*Hermetia illucens*, F. Stratiomyidae, O. Diptera) or Black Soldier Fly (BSF) is well known in USA as amongst the best biodegradation agents.

The first research program in 2005 to 2007 consisted of investigating the biology and life-cycle of Hermetia in the Indonesian climatic conditions. Many data have existed especially from USA, where BSF has considered as benefited insect which may provide many good services for humanity and environment. However, previous research before any vulgarization to the population we need a strong evidence about the non aggressively from the insect. The life cycle may be different from one place to another. A complete observation has been made on the larvae stage. The number of instars and molting during the pre-pupa stage, have been precisely established. This is a tentative method for aging the larvae stage, which may use as reference mark during the development of the larvae. Larvae aging is a bio-data need to put in relation to the larvae biodegradation activity. This is an example of some news research need that we have done mainly accomplished. Many research topics have been investigated on the adult stage like differentiation male from female or some bio parameters like relative fecundity with female body length or the correlation between eggs mass and it related numbers. All this activities have taken us for two years and the opportunity to provide the research topic for a S2 graduation at the IPB University in Bogor. Two others graduations have been accomplished on microbiology (fungus and bacteria) during the fermentation processing of PKM previously prepared before the larvae take actions. During this research period, we have discovered a biofertilizer come from the metabolism rejects from the larvae. This topic has been proposed to the laboratory of plants physiology of UI (University of Indonesia), another opportunity for a S2 graduation. All this research have put us to involve for two year. After trials in the laboratory in BRBIH Depok, the application actions have been transfer to in the BBAT Sungai Gelam- Jambi, a pre-field application. The goal was to evaluate if any harmful impacts on the environment or on the human being. The tryout for an intensive culture at BBAT Sungai Gelam has been started. During the whole year of 2007, the field test has been shown that high density BSF rearing is possible. The processes have no negative impact on the environment nor on human habitat. The BBAT became a "show room" to promote maggot for the farmers from the Jambi province and from the bordering provinces like Bengkulu, Riau. The results have concluded that Hermetia population can be domesticated to biodegrade PKM into Maggot biomass for aquaculture. On 2009, a pilot project has been decided in common agreement between IRD/BRKP/DGA, together to setting up at Singkut village as a real scale tryout "a Maggot pilot project" to learn and to reorient if any rectification to optimize. The project has run during 6 months, but a catastrophic big flood has washed 8 ponds among 12 experimented, causing a big lost for the farmers and for the project it self. Detail will be present in the report. At the same time the prices of PKM have high sky rocketed, linked with the international demand which have made the research program turn to slow-down and looking for the new alternative way to go. From where we have developed a new topic, called "Bioconversion on organic wastes other than PKM" which has been started since 2011. The test using maggot as food base on fish feeding has tried out with Red Gourami (Osphronemus gouramy). The result on their growth has surprised more than one specialist of Gourami by observing a remarkable growth within 6 months from 80g on june 2011 to 450g recorded on November 2011. The Gourami, considered as a slow grooving fish, the specie may change it reputation while cultured with live maggot as food base.

Acknowledgment

I would like take this opportunity to present my thankfulness to the Department of Fishery and Marines Affairs of Indonesia through AMAFRAD (Agency for Marine and Fisheries Research fo development – BRKP) and through DGA, Direction General of Aquaculture, for the support the bioconversion research program (financial, material and human support) during this 7 years. I would like to renew again here my sincere gratitude.

I would like to thank to IRD by extend my posting in Indonesia exceptionally up to 7 years. My gratitude goes especially to Department of Expertise and Valorisation of IRD by believing on the project minilarve and accepts to finance it implementation. By return, this is an obligation from my part to make this accomplishment with success.

The Cooperation and Cultural Action, belong to Ministry of Foreign Affairs of France (France Embassy in Jakarta) has support financially every year since 2006 the program Bioconversion. I dedicate the success of this project bioconversion today must go to SCAC support actions.

Secondly my gratitude goes to all persons below who have believed and involve in the program. Without this synergy work the bioconversion could not be raised up as it is today. My thank goes to the IRD Administration Personnel at Kemang Office.

First special thanks must go to my colleague Domenico Caruso who has accepted after my departure to replace my role as project leader of Bioconversion Program. My most sincere thanks go to:

Melta Rini Fahmi (First Indonesian Scientist has been involved in Bioconversion since 2004, BRKP, Depok), my close Colleague and Friend. I Wayan Subamia (Director of BRBIH - Depok) Rachmawati (Former personnel of IRD and My student S2) Emilie Devic (Fisheries engineer - IRD) Pascale Talamond (Biochemist - IRD) Yogi Himawan (BRKP - Depok) Ika Ayuningtyas (IRD) Irmawati (IRD personnel) Nina Meilisza (Nutritionist - BRKP) Rina Hernawati (Nutritionist - BRKP) Ibu Eny Kusrini (Researcher – BRKP) Ruby Vidia Kusumah (Research Assistant – BRKP Depok) I Made Agus WM (technical support) Urip (technical support) Kuncoro (technical support) Rusman (technical support) Yupri (Gardiner)

And Never forget the DGA Team: Pak Maskur, Ahmad Hadadi, Supriyadi, Ediwarman, Wisnu Adianto, Yudho Adhitomo,

And I apologize to colleagues and friends for whom the name are not listed above.

General reviews

1. Current and new rend needs in the Future Aquaculture

Fishmeal replacement (resources constrains)

Sustainable aquaculture is aimed at progressively replace the deficiency resources from marine and inland fisheries that have become depleted by overfishing combined with environmental changing over the past decades, and to provide such resources all year round, thereby guaranteeing the livelihoods of populations, especially in regions of the world where demography is still increasing at a fast pace. In particular, the decline of wild stocks and the subsequent collapse of fisheries also represent a threat to conventional aquaculture, which largely relies on the use of fishmeal as the main input to fish pellets. As a reminder, feeding costs amount to 60-80% of the production expenses of fish in aquaculture. Additionally, the local standard of living and the low retailing price of most fisheries and aquaculture products cannot afford those performing but over expensive feedstuffs that are developed and utilized for the culture of a few species with a high value added such as shrimps or carnivorous fish species.

Starting from this viewpoint, numerous research efforts have been dedicated during the past decade to identifying and evaluating alternative sources of proteins (and lipids) for feeding fish, in particular from vegetal basis and from sources that are abundant in developing countries in the tropical region. Several issues postponed the full replacement of fishmeal by vegetal proteins and lipids, such as the low protein content of several meals from vegetal origin in comparison to the high protein requirements of a series of cultured fish species. Additionally, many vegetal products cannot be utilized straight by fish and require further transformation. These constraints have restricted the scope of vegetal by-products to soya, cowpea, cotton seed or groundnut cake.

Apart of these few, the agro-industry produces vast amounts of by-products, which are generally unused properly or even raise pollution issues. As for other types of wastes, the stocking management of these by-products. In tropical regions, this typically applies to the palm oil industry, which produces globally 5 million metric tons of by-products each year, and to the groundnut oil industry, which apart of the groundnut cake, produces 1.5-2 metric million tons of by-products that cannot be used efficiently because of their frequent toxicity as a result of contamination by aflatoxin.

As a corollary, research orientations are increasingly refined with the scope of changing waste to wealth through biotransformation. Insects in general and flies in particular have evolved stunning, and largely unrevealed capacities of biotransformation which demand to be explored and used to the benefit of mankind.

This context was the background and the driving force of my research in aquaculture over the past 6 years, which has been focused on the development of new opportunities of valorizing the by-products from the agro-alimentary industry in general and of palm-oil in particular.

Historic context

In August 2003, a visit in France has been organized for two VIPs¹ from the Indonesian Ministry of Fisheries and Marine Affairs:

• Dr Fatuchri, Director General of the DGA (Direction General for Aquaculture). The DGA is one of the six Departments of the Ministry, in charge of aquaculture development by actions on vulgarization, promotion and extension of sustainable aquaculture in marine and fresh waters of Indonesia. The DGA combines many aquaculture stations (marine and freshwater). Sukabumi / BBAT (Java) is one of the freshwater stations.

• Dr Ketut Sugama, Director of the CRIA (Central Research Institute for Aquaculture). The CRIA is one of the three research institutes belonging to the BRKP (Badan Reset Kelautan dan Perikanan). BRKP is another of the six departments in the Ministry of Fisheries and Marine Affairs). The CRIA is involved in different research topics which could be an "upstream support research" to the development of aquaculture in Indonesia.

During the short stay of our guests in Montpellier, laboratories and research facilities were visited both in IFREMER and IRD research centers. Different research programs, not only aquaculture, were presented. As our guests visited the GAMET, I took the opportunity of introducing my research in aquaculture, and especially the current research program in the Guinea Republic as (1) rice-fish culture in the forest region, (2) fisheries management in small water bodies and (3) the valorization of agro-industrial by-products through aquaculture.

The third topic, "bioconversion processing of Palm kernel meal (PKM) and its valorization by aquaculture", represents a major interest for Indonesia. Indeed, Malaysia and Indonesia are respectively the first and second global producers of palm oil, and both ensure at least 80% of the world production. According to the USDA statistics 10.6 million tons of global palm oil, included 9.6 million tons of palm oil (PO) and 1.1 million tons of Palm kernel oil (PKO), were produced in Indonesia in 2003. The by-products (PKM) of PKO extraction amount to about 1.2 million tons.

According to the two VIPs, within the next five years, because of the fore coming plantations in Sumatra, Indonesia will become the leader in palm oil production.

The prognostic was right. Since 2008, Indonesia has became the world leader in palm oil industry by producing more than 20 million tons of palm oil per year and 2,5 million of PKM as the by-product. About 75% of the production of PKO is exported. The remaining 25 % are used locally for feeding ruminants. In term of tonnages produced, 300,000 to 400.000 metric tons remain available for aquaculture. Straight from the factory, the price of PKM is 500 to 700 Rp / kg (0,006 €/kg). Biodegradation and utilization in aquaculture could increase substantially the added value of this by-product.

Following the visit of Dr Fatuchri and Dr Ketut in Montpellier, I was kindly invited for a short mission to Indonesia in September 2003. The mission was to meet some key partners from private companies and research institutes (DGA, CRIA, Indonesian Biotechnology Research Institute for Estate Crop in Bogor) and to take advantage of this opportunity to participate to a seminar on fish feeding and fish nutrition in Bogor.

One of the focus of the mission was to investigate on the potentiality of the PKM availability in Indonesia as well as the Rubber Industry in Indonesia by visiting two factories: (1) a latex

¹ very important persons

processing plant next to Sukabumi (Java) and another (2) on palm agro-industry nearby Jambi (Sumatra), the Sawit Factory "PT. ANGSO DUO Sawit". The visit has opened the Idea how much more adequate and justified, the idea to develop the research on Bioconversion on PKM in Indonesia. Just an rough idea of comparison, only in 2003 the amount of PKM has been estimated at 1.5 million metric-tons per year, while in Guinea Republic the yearly production was around 7000 to 10.000 metric-tons.

At the end of the mission, a rapid debriefing report has been submitted to the Cooperation Service, a branch of MEA (Ministry of Foreign Affairs) at French Embassy in Jakarta), who has suggested IRD to make, in the final report, a proposal program for a financial support.

A long-term mission (four months) during 2004 was approved and supported financially by IRD in February 2004. The mission took place in between June 27 and October 27, 2004.

The purpose is to investigate the context (partnership, potentiality of by-product PKM and to confirm the interest of the program before decision of my posting in Indonesia from 2005 for two years first. The post could be extended depending on the development of Bioconversion and the interest expressed by the Indonesian Authority.

Understanding the aquaculture situation and the problems faced

Indonesia's demand for aqua feeds, specifically the commercial fish pellets, has increased due to the expansion of its rural aquaculture sub-sector. However, the price of aqua feeds or commercial pellets for aquaculture nowadays has become unaffordable to many small-scale fish farmers due to the ever increasing price of fishmeal, which the country imports from Peru, Chile, and other countries. The price of fishmeal also continues to soar not only because of its high demand worldwide but also because of the transportation cost and, above all, the stagnation of fish production from the natural resources (Naylor et al., 2000). Indonesia spends about US\$200 M per year for the imported fishmeal for mainly its aquaculture industry. If this situation continues, the development of the country's aquaculture will be hampered. Fearing further decline of fish production from aquaculture while boosting the rural economic sector to address the country's food security concern, Indonesia has tried various alternative ways to produce aqua feeds using locally available ingredients.

Basic principles of my Mission in Indonesia

IRD Mission related to Bioconversion Program - Terms of Reference (TOR) and duty.

The terms of Reference of my posting in Indonesia was established and centralized on aquaculture issues more precisely on the aqua-feeds shortage facing the lack of fishmeal imported from abroad. Bioconversion, by producing biomass of maggots (*Hermetia illucens* larvae) may play a role as an alternative and a new source of proteins to compensate the lack of fishmeal.

This is the **central guide line** from which the Research program must be designed to answer at the end, at least seven (7) main criteria as follow:

- 1. The processing method must be able to reach the capacity to produce mass amount of this new resource to respond the real need. This point must classify the research topic as an ambitious program.
- 2. The processing method must be eco-friendly that means no harmful to the environment.
- 3. The raw materials input used, must be free from competition with others foods production sectors.
- 4. The production cost of the new alternative source by itself must be competitive with the existent fish meal price.
- 5. In case of application in a rural context, the processing method must be economically sustainable as free from energy dependence and need.
- 6. In terms of safeness for the operators, the process must free from any use of chemical dangerous products.
- 7. The technology should be easy to be handled accordingly to the local population technology level.

This is a guide line points that we must be aware and keep always in mind during the research design and analysis.

Synergic Collaboration between IRD/CRIA/DGA teams and actions programs - Partnership and Collaboration based on a mutual goal of interest.

It is a perfect schema of a synergy partnership research in Indonesia in term of collaboration. The program follows the guidelines of the project Fish-Diva, which started in 2005. The cooperation is developed mainly with BRKP/CRIA in LRBIHAT Depok. The development component is realized with DGA, mainly through BBAT Jambi and BBPBAT Sukabumi. The collaboration with the BRKP/CRIA in terms of laboratory research aspects with a small scale control application and (2) collaboration with the DGA in terms of applied programs to test on the fields for feasibility control before extension to public domain.

Practically, at Depok station, which belongs to CRIA, a team of 5 persons (IRD/CRIA) has been identified to set up and to conduct the bioconversion program. At the BBPBAT Sukabumi, which

belongs to the DGA, a collaboration IRD/DGA has already started on fish nutrition issues. Integration of Bioconversion could be appreciated as a reinforcement of the existed collaboration. Later on, within the scope of the extension action, others stations of the DGA could be part of the frame work of the bioconversion program especially in Jambi province at Sumatra Island.

A common implementing, in terms of state of mind, would address to all partnership among BRKP as well as to DGA collaborations. It would be based on a franc and sincere collaboration, where the real **back bone** remains simply on a free exchange in terms of research decision, accordingly to the goals of interest dictated by the guide line of the Department of Fisheries and Marine Affairs. This free collaboration must go also to the research designs, data exchanges as well as to the participation in the research costs.

Reinforce the future capacity building,

This is a common goal of interest of IRD mission and the Indonesian Authorities Guideline. By another word, this is an act of **sharing** of experience which means for any research topic identified, the topic must be exposed to the different universities authority to involve in the research work. This collaboration as an opportunity for research topic for graduation students level S1 or Level S2 depending of the degree of research complexity. This would be an occasion to establish another type of collaboration with the University Supervisors who are looking for the opportunity to find and conduct the student work involving in applicability from research to the real Development program. The direct benefit to the student would be to learn:

- How to choose and define the goal of a research topic close to the applicability,
- How to build up the references bibliographic (using Endnotes software as a references base
- How to access to IRD Reference bases (ScienceDirect, SpringerLink and others Web of Science, Current contents, etc...) to guide the students to the modern world of research connectivity.
- How to design the research approach to answer the question goal.
- How to collect the data and to understand the validation by the statistic base analysis.

This part of duty has given us the opportunity to identify to select the good candidates to integrate in the research team in the future.

Back-ground Understanding

Principle of Bioconversion as a natural processes

BSF larvae (or Maggot) play a central role in the bioconversion process, as a bioconversion agent, during its three weeks of live time of larvae stage (from embryo to pupa stage). The larvae feed the decayed substrate, including probably all the micro organisms presents in it (essentially bacteria). The growth of the larvae generates the larvae biomass which constitutes the end result of the bioconversion process. The maggot biomass, at the final harvest, could play as a new food resource base for animals and fish. This is an example of the conversion from "Waste to Wealth" using a benefit services provided from an insect *Hermetia illucens* known also as Black Soldier Fly (BSF) during it larvae stage.

What it is all about "Bioconversion" !

Therefore, a better understanding of BSF biology and it life cycle, represents a first knowledge base, a priority goal, for a better management and the domestication of the insect for the mass production of their larvae.

Palm Kernel Meal

Palm kernel Meal (PKM) namely Bungkeal in Indonesian Language, is a by-product from Palm Oil Industry. This by-product contains roughly 18% proteins and 20% of fats. Presently, Indonesia disposes every year nearly 2 million tons of PKM. About 1/2 are exported to Europe, China and Australia with a very low price (less than 0.02 US \$ / kg). Big amount of PKM remaining in the country, still not be used appropriately. Large quantities of PKM are neglected among organics waste and may damage environment by organic pollution.



Lauric acid (CI2) ~ 48%

fly.

Bioconversion program in Indonesia

First contact with BSF

Many reasons may explain why before our work on BSF in Indonesia, the insect Hermetia illucens by itself was unknown in Indonesia because of the rarity of the insect around human habitat. The low density of the population may explained by the high rate of the predation from egg-embryo stage (ant), larvae stage until adult fly. The natural habitat of insect Hermetia is composed by dense vegetation (small bush). For sure, the people have met the BSF before but we may consider it by mistake as a wasp. And when accidently people have met the maggot (BSF larvae) they may take it by similarity as belatung (Musca sp larvae or Chrysomya sp, blow fly larvae) with a repugnant consideration.

An Open Gate

Hermetia illucens is a well known as cosmopolitan species. During my mission in Indonesia June 27 and October 27, 2004, we have no opportunity to meet yet the insect. The collaboration with BBPAT Sukabumi on bioconversion program was already started.. The PKM fermented has been prepared as media for the culture Maggot and has been placed at the back yard of the station. Only on Mars 2005, BSF populations were appeared on the old culture media, with some underform of Maggot and some others under form of old chitin skin after imago hatched.



In front of this proof, my feeling was the "the open gate" for the bioconversion program in Indonesia. After Sukabumi, it was the turn of Depok, to fine also the BSF population and to develop the first mass culture using the barrel drum.

Identification confirmed at LIPI insectarium Cibinon Bogor.

After the discovery the presence of BSF in Sukabumi and at Depok research station, our first reaction was to confirm the exactitude of



the specimens it belonging in terms of taxonomy and systematic classification. For this purpose, we have went to the Insect collection at LIPPI at Cibinon (Bogor). At the first look, by comparing with the collection specimen, it was no dough to identify as Hermetia sp. But when the comparison went further based on the



wing veins and their branch, our conclusion was clear that all specimens we have found in Depok and Sukabumi should belong to Hermetia illucens (Linnaeus-1758) (F. Stratiomyidae, O. Diptera). It was also an opportunity to access in many documents and data bases related to Insects Order of Diptera.

Vulgarizations Maggot program beside the population opinion

We were conscientious about constrains and difficulties, we would be faced by choosing to work with insect Hermetia illucens larvae. The issue would not be in term of scientific or technical point of view, but the problem will be the psychological aspects. Indonesia, similar to others part in the sub-region, is a country where the traditional values remain strongly attached to an ancient tradition believe or religious. Something new relied to an unusual practice or unfit to the local believe, would be hardly accepted.

Working with insect larvae such as fly maggot having a connotation of unhygienic dirty staffs , would face inevitably the problem to convince.

Fortunately, the project has been supported by many important Personalities, in the fisheries Department. The fact that General Director of Aquaculture has decided to support financially (200.000.000Rp) to make the research and develop of the maggot project at BBAT Songai Galam (Jambi) the decision has made a big move of the Bioconversion program to the acceptability. The Head of BBPAT of Sukabumi have another brilliant idea to baptize the call of "Maggot" to Hermatia illucens larvae, instead of Belatung that has a connotation repugnant, while the word "Maggot" is neutral (for example the giving name Jalan Maggot) do not have any special connotation. Also BSF, Black Soldier Fly become **SBF Seranga Bunga fly**. From IRD, we have promoted the keyring including together maggot and insect BSF in the same inclusion. All those actions came from a deep reflection have produce a significant effect on the opinion.

This is an example of a synergy putting together intelligence make a brain storming to find a good way to go and shortcutting the problem.

Evidence research needs for Confirmation

According to the publications on Hermetia illucens, almost all authors have confirmed that BSF insect are not classified on the insect pest list. But on the contrary BSF may provide many advantageous services to the human being as well as environment.

But despite these considerations, we are very careful to avoid any adverse consequences or any negative impacts on the environment.

That's why, although we have agreements from the Department, the spread should be forward with great care and attention. These are examples of approaches that we present later in this document on our interventions in rural areas at Bengkulu province and on Sarolangun in Sumatra.

Insect Hermetia illucens (Linnaeus, 1758)

Hermetia illucens (Diptera: Stratiomyidae) or black soldier fly, was described by Linnaeus (1758). It is a member of class of Diptera (fly) which is characterised by a pair of membranous wing on the mesothorax. While, the metathoracic wing pair is reduced become a pair of halter – wing atrophied (Figure) the two halters (white in color) are visible when freshly emerged from pupa chitin and when the wing remain crumpled. The same wings have spread after 3 mn and the two halters are covered and become not visible).



Classification and Characteristics



Diptera consists of two Suborder, i.e. Nematocera and Brachycera. H. illucens is a member of Brachycera whose has such a pair of antenna which is shorter than thorax, whereas Nematocera has a pair of antenna (usually filiform or plumose antenna) which is longer than thorax.

Brachycera consists of four infraorder, i.e. Xylophagomorpha, Tabanomorpha, Muscomorpha, and Stratiomyomorpha. Stratiomyomorpha is the infraorder that consists primarily of family Stratiomyidae and two small related families, i.e. Xylomyidae and Panthoptalmyidae.

Stratiomyidae consists of twelve subfamily, i.e. Parhadrestiinae, Chiromyzinae, Pachygastrinae, Beridinae, Antissinae, Clitellariinae, Hermetiinae, Chrysoclorininae, Nemotelinae, Sarginae, Stratiomyinae, Raphiocerinae. Hermetiinae is distinguished by the characteristic of antenna, the eighth flagellomere is elongate and thickened. Hermetiinae also does not have any scutellar spines. Genus Hermetia is characterized by the laterally flattened eighth antennal flagellomere.

Repartition and distribution zone of presence of insect Hermetia illucens

Originated from American continent, Hermetia illucens populations have been spread over the world by sea transportations. Today the specie is became cosmopolitan between 42° degree North and South (Leclercq, 1997). It is clear that the insect adapted well to all new continents, in Europe around the Mediterranean Sea in Malta Island (1926), France (1951), Italy (1954), Spain (1962). Their presence has been recorded at the Canary Islands (1972) as well as in Switzerland (1987-1988). BSF populations are found in Australia and New Zealand. In Africa, H. illucens has been recorded in Guinea Republic (2008), Madagascar (1930).

Biology learned from literature

Research on insect Hermetia illucens were studied since 40 years ago in USA (Myers et al. 2008, Tomberlin et al. 2002, Sheppard et al. 2002, Lardé 1989). The BSF populations are found naturally concentrated around garbage or dump. Naturally the females laid their eggs around compost areas; excrement of poultry of swine manures the cadaver of animal or human. According to the temperature, the eggs may hatch from 3 to 6 days. From 2 to 4 week at the larvae stage, the larvae feed non-stop during day and night. But very few information are available concerning growth of BSF larvae in term of size or body weight. No precise studies are made to investigate on the number of Instars as well as on the number of molting. Only Gerhardt (2002) mentioned that it passes five instars before pupation.

The longevity of the insect adult is estimate from one to two weeks. (Tomberlin et al. 2002, Myers et al. 2008) have mentioned that if provide water the insect adult may extend a longer longevity.

The larvae feed insatiably during 3-4 weeks depend on the fed (Tomberlin et al. 2002, Myers et al. 2008). Nevertheless, information of instar is little known. Hall and Gerhardt (2002) mentioned that it passes five instar before pupation. Prepupa will migrate from feeding site to dry and hidden site to initiate pupation. The pupae reach the adult stage in about 2 weeks (Myers et al. 2008, Hall & Gerhardt 2002).

Longevity H. illucens ranged from 1 to 2 weeks depend on larval diet. Furthermore, the adult that provided water lived longer than those not provided water (Tomberlin et al. 2002, Myers et al.

2008). End to end mating occur 2 days after emergence. Then, oviposition occur 4 days after emergence (Tomberlin & Sheppard 2002).

Sun light is determinant to occur mating. Zhang and al. (2010), has proved the artificial light, with intensity of more than 200 µmol m-2s-1 may provoque mating all year round.

A word on the anatomy and morphology aspect

The insect *Hermetia illucens* (Linnaeus, 1758) belongs to the subfamily Hermetiinae and to the family Stratiomyidae and the order Diptera. The genus *Hermetia* and the specie *illucens* is present and widespread in Indonesia (cf. Insect Collection at LIPI, Cibinon, Bogor). Four other species, also are mentioned in the territory. This is H. cerioides Walker, 1858b, H. laglaizei Bigot, 1887b, H. inflata Walker, 1858, H. rufiventris Walker, 1860b. Their presence was reported in the islands of the Moluccas and Irianjaya.

species *Hermetia illucens* (Fig. 1) is part of the natural ecosystem in Indonesia, but the insect populations are unknown by the general public. Black Soldier Fly has surly been seen but it could be taken by mistake as Wasp. As have indicated by Woodley, that most species of Hermetia resemble wasps in flight, and some species are close mimics of wasps and bees. Larvae of most Hermetia species are found in decaying vegetation, sometimes associated with a specific plant. Others are more general scavengers, such as *H*.



illucens, which has been spread throughout much of the world by commerce. It has potential as a biological waste management agent to control plant and animal waste products (Tomberlin & Sheppard, 2002 and references therein). James & Wirth (1967) reviewed one small species group of Hermetia, but most species are difficult to identify.

Morphologically, the specimens have been identified as belong to the *H. illucens* specie but furthermore, the confirmation must be based, in the future if possible, on the molecular study as Brammer and von Dohlen (2007).

Anatomy

By presenting some description on the anatomy, we have no pretention to make a complete and detail description already have been done by entomologists specialized in Diptera. We just propose to have a close up look on the morphology of two part of the insect body which may us to understand the feeding behavior and the reproduction aspects. Another word, we interest to examine closer on the mouth part and genital organs, useful to be able to recognized et make distinction between male and female specimens.

- ٠
 - The mouth part of the adult insect,

A close-up view has shown the BSF mouth part is a labial type with many micro hairs for collecting flowers pollens. No sting or bite part have existed. Morphologically, Woodley (2001) explained that



Hermetiinae closely related to Chrysochlorininae where the mouth part is a labial type.

Like blow fly, labial mouth plays as a suction sponge to absorb the liquid form of food like nectar or diluted honey. This information are very useful for us, in terms of designing an appropriate way to feed efficiently the insect BSF in case of intensive rearing in a enclose areas.



• Anatomy of the sexual organs

The purpose of the study on the differentiation between male and female by morphological criteria, is to be able to distinguish rapidly between male and female in terms of sex-ratio study or to select and pair male and female. The differentiation is based on the anatomy focused on the extreme posterior part of the body.

The male is easily recognizable by the two sharp claws acting as a pair of pincers to hold tightly the extreme part of the female ovipositor, during mating. The female is recognizable by the posterior part of the abdomen, the ovipositor extensible with at the extreme part a pair of cercas which play an important role in guiding the ovipositor to range in order the eggs. Sometime the extreme part could be retracted in the abdomen. In that case we need to, gently, apply a slide pressure on the abdomen, which expulse the extreme part distinguishable rapidly.



Research Approach to understand the pre-pupa stage

Eggs collection

The studies have been started from eggs and embryo stage of development. Adult insect imago is quite easy to to capture by a natural method. Metal drums (70-liter volume) made from barrel drum is covered with galvanized chicken wire to protect the culture from outside intruders (rats and mice) to interfere and disturb the culture. Inside the barrel we dispose 3kg of fermented PKM which emanate a



particular aroma (smell) that attracts the females of BSF to lay their eggs. The barrels are covered by corrugated plastic roofing, cut into small pieces to avoid



flood from the rain. The whole facility is placed among the vegetation (small bushes) where BSF population used to take it as refuge or as its natural habitat. Different from flies Musca (housefly) or blue flies, the BSF females lay rarely

eggs directly on the wet medium. Eggs are always deposited on substrates such as dried banana leaves place above the medium. After three days, eggs are hatching the young larvae migrate to the medium to feed and develop. The growth of larvae is very fast in the fermented PKM. Larvae during their development change 5 to 6 times their sloughed skin (molt). According to Sheppard, the number of instars or mutations is about 5 times. No matter how, the number may be considered as in the same order of magnitude.

Tentative to establish the Larvae Aging

Observations to determine the numbers of molts and instars of larvae stage of *Hermetia illucens* seem to us as a important knowledge. The purpose is at the end to be able to recognize their belonging to what instar described accordingly to their morphology. The term "Prepupa" designes the inactive period of larvae until pupa. The time where between embryo stage and the pre-pupa stage is known as larvae stage, that would end at until the pre-pupa stage. In fact the larvae stage is composed with more complexes in term of number of molting with relation with physiology of the larvae and their behavior. We propose to study the larvae and pre pupa stages with more details in terms of the number of molting. The morphometry of the larvae and their external aspects. Their composition of nutriments will be included in the study. This information could help us to understand the better moment to exploit the biomass of larvae and to optimize the preparation the pupa for the restocking purpose.

Knowing in detail the number of molting and thereby the number of Instars may give us a better reference marks to observe the behavior and the change of their physiological conditions. For example what moment of the larvae existence, occurs the maximum of their capacity of biodegrade the waste and what moment the larvae may stop feeding.

Insect Hermetia illucens, like any organisms in arthropod Phylum, is characterized by an exoskeleton made from rigid chitin. In order to growth, the organisms must change periodically the external skeleton. The term of instars mean the laps of time extended between two successive molting. Their growth may occur during each instar. Relatively to the group of insect

Holometabolous, the term instars concerns only during the immature larvae stage. The use of the term cannot be appropriated in the latest stage of pupa or imago (adult stage which does not molt any more).

To observe the exact moment of molting by detecting the sloughed skin, we have used a medium with in color. Because we have found that the color of the medium made from PKM fermented is very difficult to distinguish the molt skin. To go over, we proposed to use another

medium which is white in color. For this purpose, we have used a medium composed by oatmeal mixed with water and yeast. After 24 hours the medium is fermented and ready to receive the Hermetia eggs. Three groups of cultures (according to three dates (13th, 14th and 17th of October 2008) are considered. The culture medium is kept inside a Petri disk. Twelve cases are investigated (see table). All the culture are preserved in the lab temperature varied between 25°-28°C. The observations were done two times a day: one in the morning around 7-8 A.M. and the second at the evening around 5-6 P.M. To make easy the observations, during the control, the Petri disk are placed on an upside-down round-flat ceiling lamp. The lighting from the bottom has allowed easy the distinction of the sloughed skin (figure abow).

The results of the observations are presented below.

It seems that we have counted 6 instars within the pre-pupa stage.

Hatching : Just after hatching, the larvae, measured 0.9 mm long, migrated from substrate to the food areas. If the food sources are not available, the hatched larvae may die after few hours. They move by extending and retracting the body. The same time they moves, the larvae feed observable on the mouth part monuments.

First Molting : After 48-72 hours, the first molting may occur. The measurement of the larvae body length take place just after. It measures 1.9 mm

Second Molting: At 48 hours later from the first molting, the second molting may occur. The body length may take 3.8 to 4,2 mm long.

NB : The two first molting are difficult to follow because of the distinction difficulty of the sloughed skin mixed with media, the rapidity of the molting and the short time period of instars.

While from the third molting the detection becomes visible and easier. No pictures of the others molting is presented. The molting table below may show until the pre-pupa, pupa and emergence.









	Observations conducted on Molting of Hermtia illucens larvae																			
Hatching date & time		13	/10/	08 7:	:00 A	м		14	14/10/08 6:30 AM 17/10/08 8:10 AM											
Case N°			1	2	3	4	5				6				7	8	9	10	11	12
	1	а						1	а				1	а						
	_	а						_	а				_	a						
	2	b						2	b				2	b	х	х	х			х
	3	a	x	x	х	х	х	3	a		x		3	a				x		
		а							а					a						
	4	b						4	b				4	b			х		х	х
	5	a	x	x	х	x		5	a		×		5	a		×		x		
	c	а						c	a		^		c	a		`				
	0	b						0	b				0	b			х	x	x	x
	7	a h		×	x	×		7	a h				7	a h		×				
	8	а						8	а				8	а						
	-	b						_	b		x		-	b						
	9	b			х			9	b				9	b			х			
	10	а						10	а				10	а				x		
		b		x					b					b		x			x	x
_	11	b				х		11	b		х		11	b						
nd 0	12	a						12	a				12	a						
. 6:0	12	а						12	а				12	a						
00:9	15	b						15	b				15	b						
ing 5	14	a b						14	a b				14	a b				x		
even	15	а						15	а				15	а						
; q		b							b					b						
	16	a b						16	a b				16	b						
and	17	а						17	а				17	а						
Ę		b a							b a		x			b a			x			
00 ar	18	b						18	b				18	b						
- 8:(19	a		x				19	a				19	a						
7:00	20	a						20	a				20	a						x
ning	20	b						20	b				20	b		х				
mor	21	a b						21	a b				21	a b						
a	22	а			x			22	а				22	а		?	?		x	
		b							b					b						
٤	23	b						23	b				23	b						
atio	24	a				x		24	a				24	a						
eN	25	a						25	a				25	a						
sdo	23	b						23	b				23	b						
e of	26	a b						26	a b				26	a b						
ime	27	а						27	а				27	а						
ndt		b							b					b						
s a	28	b						28	b				28	b						
day	29	а						29	а				29	а						
ir of	20	b a						20	b a				20	b a	\vdash					
nbe	30	b						30	b				30	b						
NUI	31	a b		-	\vdash	\vdash	\vdash	31	a b	-			31	a b	\vdash	\vdash	\vdash			
	32	а						32	а				32	а						
		b							b					b						
	33	b						33	b				33	b						
	34	a						34	a		х		34	a						
		b a		x	x	pupa	\vdash		b a					b a		\vdash				
	35	b						35	b				35	b						
	36	a b						36	a b				36	a b	\vdash					
	37	a						37	a				37	a		pupa	?	pupa	pupa	pupa
	57	b						57	b				57	b						
	38	a b						38	a b				38	a b	\square		\square			
	39	а						39	а				39	а						
		b							b		DUDS			b	\vdash					
	40	b						40	b		Pupa		40	b						
	41	a F		pupa				41	a h				41	a	\square					
		a							a					b a	\square	\square	\square			
	42	h						42	h				42	h						

	1st molting
	2nd molting
	3rd molting
	4th molting
	5th molting
	6th molting
Pupa	stop moving







First tentative of intensive culture of insect Hermetia in purpose of mass production of eggs

The natural method consists of production of larvae from natural population of *Hermetia*. The spawn collectors made from drums containing Palm Kernel Meal fermented are placed among the

vegetation nearby the house backyard. The population of insects *Hermetia* existed naturally in the area is attracted by the smell emanated of PKM fermented. Only the females of *Hermetia* are attracted, not for feeding purpose but for laying their eggs on a solid substrate nearby the medium (PKM fermented). The fecundities, estimated from 400 to 1200 per spawn are correlated positively with the body length of the female (correlation coefficient R^2 =0.694). Figure below represents the effective fecundity. It means the number of eggs spawned, laid on the medium from one female. The observations continue to collect the maximum of data to reinforce the equation.



Intensive culture of insect Hermetia in enclosed environment

As we have planned, once the life cycle of has been investigated, the next step will be the tentative to reach the mass production of Hermetia eggs by controlling the whole cycle, from eggs stage until adult stage.

Therefore, the rearing method or controlled method has been tried in April 2006 at Depok research station. A large green house (14m long x 12m large x 6m high), has been setup with bamboo frame and covered by fine net (Fig.6). To simulate the habitat of *Hermetia* insect we have grown a kind of daisy flower named *Sphagneticola sp* - family of Asteraceae (Fig. 7). This management is designed to attempt to simulate the biotope where *Hermetia* population use to gathering in side.





This infrastructure provides a new possibility to make a mass production BS larva from eggs collected in the high density of insects *Hermetia* in rearing condition.

The mass production of *Hermetia* larvae from eggs has given us a new possibility to obtain any size of larvae destined to feed small mouth fishes. A new scope named "the mini maggot" has been set up as it has presented above. Many species of ornamental fishes such as as young fry of Botia, Balashark and Black ghost have been successfully tested, an encouraging results to continue the further studies.

Bioconversion as an intensification ecological an example

As we have found during 2007 that bioconversion is not limited only on maggot biomass production. It has confirmed that the rest from the process is the real biofertilizer advantageous for agriculture. This study has become a topic research for a S2 student thesis from UI (University of Indonesia). The summary of the thesis is presented in Annex 3.

Research on biofertilizer a by-product from bioconversion process



The discovery of the utility of the rest from bioconversion process as biofertilizer was unexpected. Usually when we clean and separate maggot biomass from the medium, the maggot was used as aquafeed base either directly as live food or in fresh form mixing with rice bran screw into pellets form either it may be transformed as dried meal replacing fish meal in food formulation. By separating the maggot biomass, we have usually thawed the rest of the culture in the bananas field or underneath of some fruit trees nearby. The farmers have alerted the first observation. There were remarkable differences between the groups of plants that have received the

Figure 1.- on the left side group treated with biofertilizer and on the right side control group non treated

biofertilizer comparing with the control groups without receiving it. Therefore, the first

experimentation to give evidence on the fertilizer advantage has conducted us to work on Vigna unguiculata (kacang penjang). After 3 months of culture (from February to May 2008) an evaluation has been evaluated, concentrating on plants and fruits biomass productions. The root system developments, including the rhizosphere contact surface and the number of nodules developed, have also taken in consideration. The advantages of biofertilizer have today no ambiguity. In term of dried biomass of vegetal matter (plants and leaves), there are 3.36 times more for the group which has been treated with the biofertilizer. In term of root system development, precisely at the level of rhizosphere, there are 3.43 times more. In another word, the biofertilizer has increased subsequently contact surfaces between root and soil. In term of root nodules symbiosis, the amount has recorded more



Figure 2 .- comparison of root system – contact surface of rhizosphere - and number of nodules Rhizobium and the secondary root development – nodules root on the right.

by **1.43** times, concerning the group treated by biofertilizer, comparing with the control group none treated. It is now very clear that there is a real advantage of the use of biofertilizer on plants. However many questions remain need to support by further research. On the top of the symbiotic root system nodule (Rhizobium bacteria symbiotic in fixation of atmospheric nitrogen), the investigation must go further on symbioses may develop along as mycorrhizal associations with soil fungi? And what are the origin possible of those microorganism strain?

All those question marks represent the research topics in the future concerning the microbiology aspects. At the beginning of year 2008, since the discovery of the fertilizer proprietary on the

material left over from the PKM bioconversion, the finding has putted the bioconversion program into another consideration, which recognize as an environmental friendly research program, which is justified today as a priority to encourage. Recently, published in the Seafdec magazine "Fish for the people" and broadcasting in January through some Media in France (AFP). WWF in Indonesia as well as in France have considered the bioconversion program as an environmental friendly approach an ecological intensification example

Research on microbiology

To complete the study on bioconversion process, another aspect of research approach has engaged: the investigation on microbiology aspects related with the fermentation process. The scope of work is to understand the completion link between larvae and the "medium", the role of microorganisms like yeast, fungus and bacteria.

The central "motor" of the bioconversion process is represented by the metabolic activities where revolve the exchanges and transfers of energy between the microorganism cells and the organic residues. The purpose of the microbiology approach is to try to understand the mechanism of fermentation of PKM and in order to respond to the question frequently asked about the sources and nature of maggot food, which could involve in their rapid growth? What are the roles and places of fungus and bacteria in the biodegradation process? Are there a specific species of fungus coexist with PKM?

In collaboration with University of Indonesia, two students of S2 level, in microbiology department, have been chose to take this topic under their thesis research. The first research topic concerns years and fungus species. The study concerns the identification and the dynamic population development. The second topic involves in the identification of bacteria populations (kinetic apparition and identification of dominant species). The research programs were co-directed by Dr Dibovo from University of Indonesia and myself. The research activities are occurring at LRBIHAT-Depok microbiology facilities setting up especially for this program.

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• Yeast and fungus

Concerning years and fungus, Dwi Pang, student S2 from UI, was in charge in this research topic for his S2 thesis. The first investigation to take place is to establish an inventory of the main categories of fungus existed naturally on the PKM substrate. The second investigation consists of closely following the kinetic development of different species during the fermentation. Among the present species, we have discovered at least six categories of colonies. All are under process for identification.

By following the kinetic and dynamic development during 21 day of PKM fermentation, we have remarked the dominance of three species identified two as probably part of *Rhizopus* genus, and one as Aspergillus genus. The thesis has been defend on August 2009. The study on the fungus with PKM



Figure 3 .- Basic facility and equipments for microbiology research approach, created for the occasion at LRBIHAT-Depok.

will continue. The research will scope to discover other strains of fungus not only with PKM but concern others medium base like byproducts from copra, coffee or others agro-industries residues. The final goal of research on fungus is to identify a specific dominant species, to purify by culture



Figure 4 .- Diversity of fungus variety existed among PKM substrate. Twelve (12) different kinds of colonies are isolated. Only three (3) are dominant belonging probably to two (2) species of Rhizopus and one (1) species of Aspergillus.

and conserve as the most efficient starter for PKM fermentation or others organic matters available by big quantity. The abstract is presented in annex 3 at the end of the document.

• Bacteria

Concerning bacteria, Aulia, student S2 in Microbiology at University of Indonesia, was in charge in this research since October 2008. Comparable to fungus methodology study, we process the isolation by practicing the dilution plate method. The principle of the techniques is to evaluate only



Figure 5 .- Three (3) varieties of bacteria colonies are identified, differentiated by their respective color (white, yellow and pink). A clear dominance development seems taken over probably by Coccus group.

a small portion as a relative abundance of the total cultured populations. The dilution may go down until 10⁻¹⁰ up to 10⁻¹³. The first result has shown that most of bacteria are gram stain positive. The first investigation to take place is to establish an inventory of the main categories of bacteria colonies and followed by pure culture. Three groups of colonies classified, according to their color respectively (pink colony, yellow colony and white colony) have been identified as the main group involved in PKM fermentation. Again, all are Gram stain positive. Isolation followed by pure culture has shown that all are belong to coccus group. The identification will take place by using DNA sequencing in the near future. The first comparison on two groups of culture Medias where one group has inoculated with larvae (started from eggs stage) and the second group without larvae inoculation. The bacteria densities were subsequently 3 times lower for the group inoculated with larvae. This is the first confirmation that *Hermetia* larvae may feed with bacteria developed in the media. Each treatment case has designed with replicated.

The abstract is presented in annex 3 at the end of the document

Application of Bioconversion program to Develop the rural aquaculture – Singkut Village Sarolangun Jambi (SUMATRA)

Introduction

Since 2007, after the transfer from lab research at Depok Centre (BBIHAT), to develop a mass production at BBAT Songai Gelan at Jambi province. It was clear that the maggots are safety and may be used as an alternative raw material to replace fishmeal. The year 2008 was highlighted by the success of the first trying of maggot within the rural communities. Maggot has been recognized as an easy technology to handle, free from energy need, alternative cheap food stuff for aquaculture. Maggot business has identified as potential new source for household income. At 2009 a financial strong support from the authorities (BRKP, DGA Dinas Provinsi) has focused the maggot program into a "Pilot Project", to attempt to demonstrate that Maggot is an alternative animal protein for fishmeal replacement. The application is planned to be conducted in Sumatra. Desa Singkut 1, (Sarolangun district, Jambi province) has been chosen as a base for this pilot experience.

Bioconversion in "Pilot program": Maggot as an alternative fishmeal replacement

The bioconversion program has reached at 2009 a decisive step by setting up the pilot program of bioconversion by putting in practice the principle of the bioconversion to serve the rural zones. Sarolangun district was one of those cases where aquaculture activity was closing down. The village Singkut 1, part of the district, has been chose as pioneer for this pilot example. What concept and approach guide line be judged as appropriate to be adopted for the project ?

The meeting held on the 4th of March 2009 at Jambi, has discussed on the appropriate design and method be used in the project. The meeting has coordinate the roles and actions of each party involved (BRKP, IRD and the DGA/DKP Dinas of Jambi). All parties are agreed on the general concept: the Pilot Project must go beyond the technical aspect of producing maggot. The Pilot project must act as a demonstration over the whole transformation chain from maggot up to fish production as the final focus. The final conclusion must demonstrate that **maggot is a new source of animal proteins capable to replace fishmeal and to provide low cost aquaculture**. Therefore the project must cover three operations steps by starting (1) from maggot culture followed by (2) the pellets fabrication based on maggot biomass. The project will continue up to (3) fish culture and harvest. Each step must take in account the economic aspects accounting all costs involved (investment, infrastructure, running cost,...). On the other word, the frame of the project may be presented as a chain of three specific groups of operators:

- The first group or Group A, represented by 5 families, are in charged on maggot biomass production.
- the second group of operators, Group B, represented by only one family, is in charged on aquafeed pellet production. The group B will buy the whole biomass of maggot produced from group A, to be used as the main ingredient inputted into the pellets composition, instead of fishmeal. The pellet produced will be free from fishmeal.
- The third group, Group C is composed by fish farmers practicing aquaculture in ponds. They will buy pellets from group B for growth out their fish in respective ponds.



Singkut 1 village has been chosen as pioneer to act in this program. The families involved in the operation are identified and decided by farmers association (Kelompuk). The agreement has also decided on the duties and responsibilities of each party as shown as follow:

- 1. The duty under IRD responsibility is to set up 2 (two) maggot culture sites (two families among the 5) and to build one storehouse for stocking Palm Kernel Meal.
- 2. Dinas Perikanan of the Jambi province is in charge on the construction of three other maggot culture sites (three families). The duty of Dinas will provide also the PKM (90 tons) and will insure it transportation from the factory up to storehouse at Singkut 1.
- 3. The duty part of BRKP is to ensure the running costs between three groups of operators: maggot producers (5 families), aquafeed maker (1 family) and fish-farmers (12 families). The running cost concerns firstly the maggot purchased by the aquafeed maker, and secondly it concerns the fingerling cost for the growth out operation in the 12 families.

PKM supply issue

After a long negotiation with Smart group, an agreement on PKM has been fixed for 200Rp/kg limited at 90 tons of PKM needed during the pilot period. The construction of the facilities needed (Maggot culture pool, stocking house) were build in time March 2009 (see report-Appendix 7). The maggot production has followed a month later. To produce massively the larvae biomass, we do not process by depending on the spontaneous spawns from the wile insect population. Thereby, we have processed by an artificial restocking by introducing since the beginning; a substantial quantity of pupa (50kg of pupa) has been brought from BBAT of Songail Gelam (Jambi). By increasing the insect breeder's population by restocking the pupa, we have recorded an immediate high production of larvae. Nowadays the harvests, between 1000 kg to 1250 kg of fresh maggot, are constantly recorded every week from the five families. The transformation into aquafeed is made in the same area, in charged under one of the 18 families. The pellet price made from Maggot base (3500Rp/kg) is much far cheaper than the commercial standard pellets (7000Rp). A show case seminar recently occurred on December 2009 at Sarolangun was organized to communicate the intermediary result and to open dialogue between the farmers and the

organization team. The pilot project will run for two cycle of fish production. The growth of fish and the Food conversion rate (FCR) will be presented around the middle of this year 2010.



Conclusion

The first analysis of the pilot program, after 9 months running, has concluded broadly optimistic results: (1) mass production on maggot is clearly feasible, although there are some refine correction to increase and stabilize the yield og maggot harvest. However, the conversion ratio has been notified between 2,5 to 2,8 (from PKM to fresh maggot biomass). The real productions have been recorded between 150 – 230kg from 450kg of PKM input. For the first time, it possible to produce, in the rural condition, the aquafeed pellets, based on maggot biomass. The use of maggot pellet on fish culture is being operated. The growths recorded by intermediary samplings are optimistic. The final result will be analyzed around May this year.

Although the positive results of this first prototype, some technical and some economical considerations are nescesserly to revise:

- The cost of prototype of the four culture basins estimated around 15.000.000 Rp per production unit (per family) is unaffordable by the rural farmers. It is advisable that technical research should focus on others alternatives cheaper materials, by keeping in mind: the efficiency of the structures in terms of maggot productions. The design must keep in account the duration of the structures lives and therefore the depreciation economic impact on the maggot production cost.
- 2. It is clear that the maggot biomass is a good candidate product for fishmeal replacement. However, the prices adjustment are required to be considered equitably at each level of operators (maggot producers, aquafeed based on maggot price and fish farmers)
- 3. For the moment the PKM price is fixed at 200Rp/kg which is a good base to start the project. But for the further step of development, negotiations must be previously agreed with the PKM producers. Maggot should be recognized as a symbiosis activity among the oil palm industry.

"Minilarvae" a second aspect of application of Bioconversion Research

Beside the "Pilot Project" a second application has been considered and named "Minilarvae". The operation concerns the mass production of small size maggot (from 1.5 - 2mm, 3mm et 4mm of diameter) will be used as live food for aquarium fish. The program action has occurred at Balai BRBIHAT Depok. This report, will presented the coordination of my schedule work in the pilot program

As have been mentioned in the introduction chapter, the research on Bioconversion of PKM by insect *Hermetia illucens*, have guided us to understand it life cycle, it biology characteristics related to the feeding habit, fecundity, reproduction behaviors, the growing conditions during the larvae stages or behaviors and reactions with physical parameters like light, temperature or color. All those knowledge notified from many observations, have been exploited and applied into two benefit services: (1) Maggot as a fishmeal alternative (as stated above) and (2) "Minilarvae" used as live food for aquarium fish. The Minilarvae terms has been adopted to define the small size maggot homogeneously regrouped into 4 categories determined by the body size diameter from 1,3 - 2 - 3 - 4 mm. Another particularity related with the minilarve is their origin, where all are synchronized hatched from the same cohort of eggs its self have been spawned on the same day. Thereby, the key solution is to produce a masse quantity of eggs by rearing the insect in an enclosed area. The technical method to obtain the difference size of maggot (minilarvae) corresponding to the mouth diameter of fish.

The process, are based on optimizing the sufficient quantity of food for the larvae and equilibrate according to the size demanded by the market. The knowledge of technical process has been protect by an international patent (Brevet PCT/FR2009/050592 submitted on the 7thof April 2009) and also under Thailand patent (brevet en THAILANDE n° 0901001753 submitted on the 20thof April 2009) because Thailand is not part of Patent Cooperation Treaty 1970 ("PCT"). To consolidate this common discoveries (Maggot and Botia) a "co ownership" document is actually under preparation to be officially signed between the French and Indonesians Research Institutes early this year (March 2010).

The minilarvae program (Minimaggot) at 2009 is highlighted by a strong support from IRD to push the project into a real scale. A budget of 45.000€ (forty five thousand Euros) has been agreed to support the construction of set up a prototype of Insectariums (1000m²) for trying a high density culture of Insect *Hermetia illucens*. The objective is to produce and harvest massively eggs from Hermetia to be used produce industrially the mini-larvae to satisfy the ornamental fish market. On December 2009, after the inauguration 05/11/2009, the production maximal has reached 300gr of eggs per week.

To promote the two products (minilarvae and Botia), BBIHAT and IRD team has participated at Aquarama exhibitions held in Singapore 26-30 May 2009. Surprisingly, the minilarvae was well accepted. About 20 countries are interested on importing the minilarve. The first sample of minilarve will be exported for trying in those countries. On April 2010, a showcase will occur at BBIHAT – Depok.



Patent





Co-Ownership agreement signed on 16 12 2010 in Jakarta

Bioconversion of organic wastes other than PKM (Palm Kernel Meal), in Indonesia

Saurin HEM (Senior scientist IRD) & Emilie Devic (Fisheries engineer, IRD)

Back Grounds and research scope

At the beginning, the motivation on the bioconversion research program was designed in an attempt to identify new sources of protein alternatives to fish meal. This research topic is now becoming a necessity. Indeed, the global shortage of fishmeal aware since 15 years has become nowadays a real issue leading to dramatic consequences for many countries where aquaculture is a traditional activity which provided an important part of the country's food security. This is the case of Indonesia where the increase in the prices of fish feed, caused by the shortage of fish meal, resulted in a decrease or even a cessation of this activity foster in some rural areas. Without an alternative to medium term, the situation could become a problem of food security for the population of remote areas. Since 2009, the bioconversion has been demonstrated for 10 months in the pilot project in Sumatra, the result is clear that it is a possible solution to reactivate.

The bioconversion is a natural process using the ability of insect larvae *Hermetia illucens* (Diptera, Stratiomyidae, cosmopolitan) able to biodegrade palm kernel meal into a biomass of larvae used as the base component in feeds for farmed fish. A simple process without the need for energy has been demonstrated in a rural zone setting in the province of Jambi (Sumatra). The only essential input is the palm kernel meal (PKM) that the project is purchasing from the factory of Smart Group at Sarolangun (Jambi province, Sumatra) near the site of the Pilot. However, the flip side of success is the speculation of PKM at this new opportunity to enhance that by-product. Result in a 5-fold increase of PKM from its initial price pushing up to 200 Rp / kg to Rp 1,000 / kg. What made the temporary halt of the project.

In order to deal with this new situation, we have chosen to go to other sources of organic waste, an alternative solution for areas without PKM.

Intensive of the insect *Hermetia illucens* rearing to obtain the mass production of eggs

Obtaining of egg masses of the insect *Hermetia* is "key" of the bioconversion process. The population density present in the natural environment, could never meet demand when needed to biodegrade a large amount of organic waste. An intensive rearing of insect Hermetia is the only way to obtain large amounts of egg masses. To achieve this, a research program to optimize both the quality and quantity of insect breeders was considered. The program may be summarized in three stages as follows:

- Getting to know the life cycle during the larval stage
- Optimize a growth in the best condition of larvae (no interruption of food supply).
- Follow the growth of larvae ensuring good condition of fermentation of PKM (moisture from the culture medium, good growth of fungi and yeasts)
- Enrichment of larvae during the second week by fish waste silage.

- Care with precautions during the development of pupae stage
- Transfer of pupae (the right time of collect and transfer, handling with precaution,...)
- incubation of pupae in puparium.
 - Precaution and care of adult insects
- Feeding insects
- Container-spawning (location and position) and technical collection of egg masses
 - Results of productions:

The curves below (production curves and trend lines in red) represent the weekly production of biomass of eggs (in grams) in our experimental structure in Depok, knowing that 1g of eggs may contain 35,000 eggs. Our goal is to be able to produce 1000 g of eggs per week or 35 million eggs



Our goal is to be able to produce, continuously around 1000g/week, equivalent to a potential output of 2 to 2.5 tons of fresh biomass of larvae produced from 6 to 7 tons organic waste. A significant new source of food for animal and for fish.

Collection of eggs Hermetia illucens 11/07/2011. 624g harvested biomass is equivalent to about 22 million eggs.



Bioconversion of organic wastes other than the PKM

Since November 2010, a production technique massal Hermetia eggs (several million eggs per week) has been developed. This possibility has opened a new perspective geared to recycling organic waste, other than palm kernel cake the PKM. The commitment in this way is first to diversify sources of bioconversion making its feasibility to other regions without PKM such as the island of Java and elsewhere. A second point of interest of this approach is the development of organic waste while avoiding adverse impacts on the environment in the absence of prior treatment.

Wastes have been tested so far under the program, are:

- Waste from fish market (trips, guts, head, ...)
- The rest of coconut milk after extraction of coconut pulp,
- The rest of the production of Tahu
- Waste from the shelling plant crab

Waste from fish market:

The Fish Market at Depok Kota may provide fish waste, composed by fish head, hoses, ... about normely 200 Kg per day. The waste may increase up to 400kg on every Saturday. This waste, without treatment may become a problem because of their odor, after their rapid decomposition under the sun. This could become a source of organic pollution to the environment. Normally, the wastes have no economic value. However, to organize it collects may cost 50.000Rp per 100kg wet weight.





Digester (concrete tank-image left), with 3m in diameter and 0.6 m high wall which contains about 36 million by larvae capable of biodegrading 50kg of fish waste in 4 to 6 hours. Right image: close up view of the larvae, which by their small size, can get into nooks and artifacts (here - the heads of shrimp) to clean all organic material in it, to generate their own biomass.

This is an opportunity to create meaningful jobs for the small community. The bioconversion of these wastes can be made directly by putting the biomass waste in a digester in which there are

millions of larvae of the insect Hermetia ready to biodegrade. The speed of biodegradation depends on the proportion between the amount of larvae available and the volume of waste to be treated. However there are two uncertain facts that may disrupt the process: (1) delay of supply of fish waste or (2) delay of the larvae preparation, those two imbalances may cause the bad fermentation. To anticipate these uncertainties and to remain independent these uncertainties, we have opted to chose to process by the method of fermentation by lactic acid silage.

Ensiling process of fermentation lactic acid

The advantage of silage is to create an acidic medium by using the organic acid (formic acid) is to prevent fermentation by bacteria dangerous unfit for larvae and causing bad odors. Indeed, the acidic medium prevents the development of a wide range of dangerous microorganisms. The medium allows to develop only two dominant groups of non-pathogenic bacteria: family of Lactobacillacae and family of Streptococcacae.

Fermentation by lactic acid may play as a storage and preservation for several months without worrying about the shipment of waste volumes or volumes of larvae to develop. This buffer capacity gives us an extreme flexibility in managing the process of biodegradation. Preparation consists of :

• have several blue plastic drums of 150 liters in volume (about fifteen in this case) empty tank ready for use as silage containers.

• 3 to 4 cans of 25 liters of formic acid ready to serve

• A metal rake and a wooden paddle, a bucket of 50 I to serve as a stepping stone mix

• As a safety measure, it is important to handle with a pair of goggles, a pair of gloves, boots and rubber apron, serving a precautionary basis in all manipulations with acids in general.

The amount of formic acid to be used by the volume of waste to be treated is 3%. It is easier to mix by small quantities in a basin of 30kg, making sure to add the acid slowly in the waste and not vice versa. Shake well all by using a wooden paddle.

Once thoroughly mixed, the mixture is poured into the blue drum 150 I. We can preserve the proteins and fatty acids for several weeks or even months. It is important to control the pH of the mixture to keep it at around pH 4.



 formic Acid by container of 25kg
Blues plastic drums of 150 litres
NB.- to mixte the 3% formic acid, we must put the formic acid after the fish waste.

The crop is to separate the larvae of debris and impurities. The biomass of larvae will subsequently be used as live prey for fish or processed into flour for use as a component in the food base for fish instead of fish meal.

Analyses of larvae enriched

Among the elements of analysis we are interested in the first place the forms of fatty acids accumulated in the larvae. By comparison, the results of analysis can reflect the polyunsaturated fatty acids from fish silage to the larvae. For this, purpose, we compared the results of analysis of two groups of larvae. In the first group, the larvae are fed without any addition fermented PKM
	РКМ	Mag PKM	silage	Mag Silage
Σ Saturated FA	63,59	43,78	44,45	31,57
$\boldsymbol{\Sigma}$ monounsaturated	10,01	19,27	15,78	18,19
Σ n-6	0,02	0,05	2,47	1,73
Σ n-3	-	0,13	12,67	6,77
Σ Others Polyunsaturated	1,90	4,09	5,31	6,92

(PKM Mag - Maggot PKM). In the second, the larvae were made from the fish waste silage (Silage Mag - Maggot from silage).

At the first place, we can see that the composition of the larvae is highly dependent on the nature of the medium. This observation has already been notified by a team of researchers in the U.S. state of Georgia. This similarity between the larvae and their culture medium is an advantage to adjust the desired composition of the larvae in using them as live prey for feeding pets (live Pet Food) that the requirement in their nutritional need can be difficult to answer in the formulation of pellets. This flexibility may add another important point in the list of benefits attributed to the larvae of the insect Hermetia.

The graphs (rada curve) below illustrate perfectly the similarity of the larvae (red line) and medium (blue line). We chose two opposit examples of the medium, one of which is very low in omega-3 polyunsaturated fatty acids (Σ n-3) and Omega 6 (Σ n-6). That is the case of PKM is the (lower left) and the other on the contrary, is richer, it is the fish waste silage (below right). We can see that the polygons, blue and red are practically overlapped.



Using enriched larvae for feeding Red Gurami Padang

The Gurami is a species of fish highly appreciated by the people in Indonesia. Known as a kind of very slow-growing fish, the Gurami is still absent from the list of freshwater aquaculture species in Southeast Asia. Yet its economic value is ranked among the highest in the market. The attempt to raise this species was first to trace the transfer of essential fatty acids first fish silage larvae and larval fish. The experiment is currently underway. But already, we are all surprised by the growth rate of guramis fed with live larvae. The completion of the manipulation is expected in late November. If the growth of these guramis could reach average weight of 400 to 500g within five months, the experience would be an achievement that could bring a different perspective on the species in association with the larvae of the insect Hermetia.





Gurami Merah 07/7/2011 (60,4 g)



Gurami Merah 23/8/2011 (140 g)



Gurami Merah 20/9/2011 (190 g)

Biodegradation of the by-product from Tahu (Ampas tahu) and the rest of Copra

In addition to fish waste, two others organic wastes were investigated. Coconut milk is an ingredient widely used in cooking traditional Indonesian recipes. As the fish waste, the by-product from copra (BPC) has no significant economic value. However, the collect from the markets or from restaurants require an organization and need some labor cost on collecting and transportation up to station Depok BRBIH in our case. The global cost represents Rp 10,000 for a 30kg bag. Compared to the price of PKM, the copra is estimated at half the price. The only constrains is the variability of its availability. But we believe that constraint could be resolved with a more structured organization.

Another type of waste is very common in Indonesia is the by-product of the production of tahu "Ampas tahu". In the manufacture of Tahu, the residue represents about 30% by weight of raw material. The Ampas tahu is often reused as an adjunct in the food animals (especially poultry). Protein content of about 20% but the fiber content is too high, a real barrier digestibility of monogastric animals in general. The price is still reasonable (1000RP/kg dry weight). This product deserves to be included in the trials of valuing by bioconversion.



Manufacturing Tahu with the residue (AMPAS tahu - pictured right) is about 30% by weight of raw material. AMPAS tahu is often reused as an adjunct in food animals (poultry and pork). Protein content of about 20% but the fiber content is too high, a real barrier digestibility of monogastric animals.

meal and the expensive cost of feed in this region of Central Java.

The bioconversion of these two products has been conducted in the study of a student Novia Bagas, a topic research to obtain a graduate level S1 from the University of Yogyakarta. The reason to work on copra and Ampas Tahu in his study is due to the availability in abundance of these two products in the region. In the case of a positive outcome of this attempt, the bioconversion could play an important role and an alternative solution, to solve the shortage of fish

Very different from the PKM, these two residues are wet to 90% water. Before any action processing, a drying procedure is required. It was found that compared to the PKM, these residues are poorly inoculated naturally with microorganisms (fungi yeast or bacteria). This observation leads us to use of "starter", composed of fungi and yeast that have developed during the

fermentation of PKM. A comparative experiment between two groups of the same residues but treated according to two types of treatments (with and without starter). The first group represents the residues were no starter has been applied. In the second treatment with starter was applied at 10% by weight of residues used in fermentation. To make easy to evaluate the difference, we have, in both groups, inoculated 10g of micro-larvae (an very early stage of larvae, one week after hatching). After the seventh day of incubation, the biomass of larvae was evaluated. The final conclusion was clear : the group for which the fermentation was started with the starter, the development of biomass of the larvae is 3-4 times higher.



The conclusions can be drawn from these observations:

- The potential of Tahu seems higher than coconut

- The "starter", by it importance must be considered in the future as a research topic to identification of the fungus, yeast and bacteria including enzymes and their effects.

Bagas Nevia work is being completed. Upon his return to his province, the contact must continue to develop in this region of Central Java, where fish farming of freshwater species is part of the tradition.

Conclusions recommendations:

The advantage of this program is now recognized far beyond Indonesia. Among the countries nearby we can enumerate Singapore. A group of cooperative (SMAC - Singapore Marine & Co-operative Association Limited) has expressed interest to partner in its program of integrated agricultural development where all waste will be recycled and recovered on site (Pilau BULAN).

The island of Reunion, a consortium group Qualitropic, Unima, Nutrima and CIRAD is being setting up progressively. The project has a goal to biodegrade organic waste from agriculture, Tuna industry and animals husbandry available in the island. Hermetia illucens exists locally. The biomass of larvae will be used for livestock and fish farming. The project plans to extend its impact to Mayotte Island and Madagascar.

Zoe-Biotech (France) company, based in Marseille, wants to develop the same type of service in France: biodegradation of organic waste by bioconversion. From 1 January 2011, a national decree on the duty applicable to all canteens (schools, hospitals, ...) in France, treat organic waste will become an obligation. The insect Hermetia exists in France, in view of these new decisions of the Grenelle Environment Forum, the bioconversion should have a place and an important role to play.

In view of numerous requests for collaborations with our expertise it is clear that the interest to invest our efforts in research on BSF is obvious. The program bioconversion, now, will be renewed by Domenico Caruso. I am delighted and wish "good wind" for the new mixed BRKP / IRD and full of success.

An unexpected Recognition

Recently, I am honored by having been selected as winner of 2011, awarded a Medal of Vermeil, a French National Award offered by the Agriculture Academy of France related to my research approach during the whole 36 years of my career in IRD. My works have been classified by the Academy as "an approach for sustainable aquaculture with a strong concept of ecological intensification".

Bioconversion is part of examples of "an ecological intensification" concept was recognized by its four benefited functions:

- 1. Valorize the organic waste " from waste to wealth"
- 2. Avoid in the same time the environment pollution from those organic waste if they are not treated
- 3. Provide a "new food resources", an alternative solution to respond to the food shortage in the near future
- 4. The by-product from the bioconversion process is a rich compost can be used for bioagriculture

I would like to dedicate this Distinction to all of my team in Indonesia and close colleagues in Depok that I have collaborated during this 7 years. This recognition is part of our common effort and successful story.



Ceremonial Solemn at the Academy of Agriculture of France, in Paris 8ème – on the 5th October



A brief speech after medal received



Heads and teals of the Medal.JPG

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ANNEXES

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Annexes

- 1. Annexe 01 .- Terms of Reference of my research program on Bioconversion in Indonesia 2006
- 2. Annexe 02 Bioconversion of palm kernel meal for aquaculture: Experiences from the forest region (Republic of Guinea).
- 3. Annexe 03.- Valorization of Palm Kernel Meal via Bioconversion: Indonesia's initiative to address aquafeeds shortage
- 4. Annexe 04.- A good example of successful bioconversion SAS IRD magazine
- 5. Annexe 05.- Maggots From Palm Kernel Meal (PKM) to Minilarvae Biomass, via a natural process : Bioconversion. Aquarama Singapore 2009.
- Annexe 06.- Valorisation 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 for ICOPE – Bali 2007.
- 7. Annexe 07.- Report on the establishment of "Bioconversion" pilot project at desa Singkut 1, (Kebupatan Sarolangun Jambi Sumatra). 2009.
- Annexe 08.- Madagascar Mission Expertise Report. by Saurin HEM (IRD/BRKP Indonesia). Proposition of a tryout on Fishmeal Replacement by Maggot-meal applied with shrimp culture (Mission made from 16th to 30th of October 2010)

TERMS OF REFERENCE

Title:

Bioconversion of byproduct from Palm Oil Industry and from others agro-industries in Indonesia for aquaculture purpose

Name of the consultant:

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Specialization:

Tropical Aquaculture and Bio-system

Period of Research program:

February 2006 - December 2007

Background and economical issue:

The world aquaculture development today has been faced with increasing price of pellet. Fish meal price is for a large part responsible for the rising cost of pellet. Indonesia has expended yearly 200 millions of US dollars on importation of fishmeal from abroad. The situation could be worse in the near future with the increasing of fuel price and the rising of fishmeal demand. Fishmeal replacement research program become now a day a real necessity to keep growing aquaculture development. The bioconversion of palm kernel meal PKM¹ for aquaculture purpose is part of this scope. The insect larvae (from *Hermetia sp.* Stratiomyidae, Diptera, also known as Black Soldier larvae) are used as the bioprocess agent responsible for digesting the byproduct. The larvae biomass generated from bioconversion are destined to be used as feedstuff for fish.

This bioconversion process on PKM has been used for the first time in Forest Region (Rep. of Guinea, West Africa). But the process become more interesting when applied in Indonesia. Second world leader, after Malaysia, on palm oil industry, Indonesia has produced yearly about two millions metric-tons of PKM. But from year 2010, because of the development of palm oil Industry will rising up in Sumatra, PKM production in Indonesia will reach 2,5 to 3 millions tons per year.

Up today, PKM has been exported with a very low price (less than 100 Rp). An appropriated use will optimize a better value added to PKM. This new finding will change the consideration of this byproduct and it commercial issue in the future. The situation could change the dependence of Indonesia from importation of fishmeal and it could open a new strategy for aquaculture development in the country.

Objectives of short and long terms issue:

• Investigation on PKM price, production and distribution circuit

Normally, we may imagine that the crude palm oil (CPO) and palm kernel oil (PKO) both come from palm fruits may be extracted and produced in the same factory. But in practice, the major factories of CPO (red oil extracted from palm fruit, drupe

¹ known also as palm kernel cake, PKC or Palm kernel expelled, PKE

mesocarp) are established within the palm plantation areas. While the kernel extracted from the palm seeds are almost all transferred to another factory where PKO are extracted. For the practice reason, related with exportation facilities, PKO factory are established nearby the port areas. The Palm kernel meal (PKM) generated after extraction from kernel, the palm kernel oil PKO, are therefore available the most at Palembang, Lampung or Medan.

How to access to PKM? Who to contact? How much it real cost? Many information and data on PKM remain unclear. To be able to organize the production for the real scale, and to make an appropriate economical analysis, investigations are required in order to understand the distribution circuit, the production data and to know the real potential.

• Research on life cycle of *Hermetia* – Black soldier

Research on BS life cycle will concern the larvae stages (timing of development from eggs to pupa stages. The study will continue on the adult phase in captivity. The biodiversity of BS combined with other insect communities associated during the culture are required. The studies will concern (1) the systematic and classification knowledge of those insects involved and (2) the dynamic of those insects population's development in the culture.

• Technical research on bioconversion processing method:

The knowledge on culture method, number of larvae instars, duration of the larvae development, are needed. Research programs will continue on pupa stage, adult life including feeding behaviors, reproduction and spawning mechanism. Investigation on population dynamic: growth, cannibalism (intra or extra-specific) are also focused. Research would scope on optimization of the conversion rate from PKM to obtain BS larvae fresh weigh, after 4th week of culture. Research will concern on enrichment technique: with different mediums, during the 4th week of culture. Investigation on nutritive elements: proximate analysis and amino acids as well as fatty acids profile in BS larvae will be concerned. This approach investigates also on the different types of mediums (for culture and for enrichment) before and after processing.

• Feeding test with divers species of fishes (digestibility survey and growth monitoring)

Feeding test has been tried with several species of fishes. Many others species (ornamental and species for consumption) will be tried following by monitoring on the growth and the conditions factors of fishes. The impact on coloration brightness expressed from the medium enrichment will be tested on ornamental fishes.

Among the fishes for consumption, the scope is to focus on carnivorous fishes which are difficult to feed with pellets. This trial will concern Ikan Betutu (Oxyeleotris) or ikan Toman or snakehead (Channa micropeltes). The test from the research scales will apply into the farming exploitation following with economic analysis.

Application to massive productions – Implication of home industry model

BBAT of Sukabumi is the research station where results from Loka Depok are applied into a bigger scale. Applications on feeding with fishes and mass productions of BS larvae remain in the previous research frame work before transferring into the village or farmer scale. Establishment of the linkage between small scale home industry and the big scale production of BS larvae will be planed and tested.

BBAT of Jambi will be the main research base for Sumatra. The cooperation between Jambi and Depok station will start from February 2006.

Investigation on bioconversion using others by-products

According to the first test on the biodegradation capacity of BS larvae, we have notified that BS larvae can digest the most organic matter wastes and can extract high value of nutrients as proteins and crude fat. By looking for the most appropriated by-products (high availability, low cost production) we have found and considered for instant two by-products locally available: (1) the seed from *Hevea brasiliensis* (28% CP and 40% CF) and (2) the byproduct from coconut after extracting Coprah oil (20% CP and 28 to 35 % Fat Acid). Therefore, both will be used in bioconversion by BS larvae.

Expected output and results:

- Investigation on PKM price, production and distribution circuit of PKM will provide for each zone in Sumatra the potentials for BS larvae production and therefore the potential for aquaculture related with the availability of this new sources of aquafeeds.
- Knowledge on life cycle of BS (larvae and adult) will provide technical approach for BS larvae rearing and its mass productions. Different dimensions and morphology of BS larvae with relationship with their growth age will permit to produce BS larvae as a life food according to the size and age of the major commercial and ornamental fish.
- Analysis on nutrients in BS larvae by proximate analysis and its profile on amino acids and fatty acids.
- Optimization of conversion rate from PKM and others by-product to BS larvae production.
- Role of BS larvae in Fishmeal replacement program. Determination of different percentage of the incorporation between fishmeal and BS larvae.
- Digestibility and growth rate of fishes feeding by BS base.
- Economical analysis and identification of constrains when apply into big scale production.

Operations schedule in different sites and team

- DEPOK Research Station (LOKA RBIHAT-BRKP) 2004 2007
 - Basic research to focus to understand life cycle of Hermetia illucens
 - Duration and number of instars of larvae and adult stage
 - Culture method, harvesting technique and conversion rate
 - Nutritive values and test on digestibility with different species of fish
 - Bioconversion from other mediums than PKM (Coprah, Ampas Tahu, Hevea seed and others organic wastes....)
 - Facilities and structures in Depok station

- Team and persons involved : Saurin HEM (IRD) & Rini Fahmi (BRKP), I. Wayan Subamia (BRKP), Yanti Suryanti (BRKP), Yann Moreau (IRD) and 4 students from University of Indonesia
- BBAT Sukabumi (DGA) 2004 2007
 - Applied research on mass production of Hermetia illucens larvae
 - Processing technique for mass production
 - Test digestibility with different species of fish
 - Bioconversion from other mediums than PKM (Coprah, Ampas Tahu, Hevea seed and others organic....)
 - Team and persons involved: Saurin HEM (IRD), Maskur (DGA), Ahmad Hadadi (DGA), Herry (DGA).

• BBAT – Jambi, Sungai Galam (DGA) 2006-2007

- Applied research on mass production of *Hermetia illucens* larvae from only PKM
- Perform the mass production technique
- Test digestibility with different species of fishes presented at BBAT Jambi
- Bioconversion from PKM and may be Solid sludge, both by-products from Palm-oil agro-industry.
- Team and persons involved: Saurin HEM (IRD), Supriyadi (DGA), Ediwarman (DGA), Rina H., Yudo A.

Budget allowance per annum

Total:			9 000 e	uros
	•	Publications (posters designs, scientific papers)	.1 000 e	uros
	•	Field trips and missions	.1 000 e	uros
	•	Structures, equipments and facilities	.4 000 e	uros
	-	Analysis cost	.2 000 e	uros

Depok, 3rd of February 2006

Ir Wayan Subamia Msc Director of Loka RBIHIT, Depok Ir Saurin HEM Msc IRD Fish-Diva Program Full Length Research Paper

Bioconversion of palm kernel meal for aquaculture: Experiences from the forest region (Republic of Guinea)

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Proteins and fats locked in palm kernel meal cannot be used directly by fishes. Enzyme from maggots (*Hermetia illucens* larvae) lyses and extracts those nutrients which are consumed as food. Biomasses of maggots produced can be used to feed fishes. This innovation opens a new hope for many African countries where so much agro-industry by-products exist such as palm kernel meal, groundnut meal, cotton seed meal. The different aspects involved in this discovery are explained before the sequence of processes up to final discovery is described.

Key words: *Hermetia illucens*, black soldier fly, palm kernel meal, rural aquaculture, agro-industry by-products, fishmeal replacement, bioconversion.

INTRODUCTION

Searching for new aquaculture feedstuff, other than the usual commercial pellets, means looking forward to solve the problem faced frequently in the African rural context, where feedstuff supplies are difficult. The high cost (including transportation) of fish pellet and its ingredients such as fishmeal, fish-oil, soybean, etc. constitutes a real constraint for the development of aquaculture. The idea to apply acadja or brush-park fisheries in brackish water lagoon (Hem and Avit, 1994) as an extensive aquaculture system (without feeding with pellets) is one of the promising responses to this rural aquaculture constraint in West Africa. Research programs to develop rural selfsufficient technology are becoming an urgent need in order to save rural life from the impacts of globalization such as energy dependence or other raw products importation.

The Forest Region of Guinea Republic is a land locked region (Figure 1). Nevertheless, the economical exchanges as well as food supplies have existed traditionally with coastal regions of Liberia and Sierra Leone. However, the situation has changed to nearly collapse since 1990 when the civil war started in Liberia and since 1997 in Sierra Leone. As a consequence of the migration of the refugees, the population in the Forest Region has almost doubled within a short period of time, increasing from 1.3 million to 2 million people. As a direct consequence from those circumstances, there is a real critical food security issue. Considering the consumption rate of fish as example, the rate was as low as 2.5 Kg/capita/year while at the same time in the coastal region nearby Conakry the rate has remained acceptable at 15 kg/capita/year.

When the Institut de Recherche pour le Développement (IRD) in 1999 initiated a research program to develop aquaculture in the Forest Region (Republic of Guinea), self sufficient concept was declared firmly as terms of reference of the project. Acadja-bamboo in ponds (Hem et al., 1995), applied from Ivory Coast experiences, was introduced. Rice-fish culture, inspired from rural practices in Southeast-Asia, was also tested (Ali, 1992; Chapman, 1992). All these new techniques may improve fish biomass, but in terms of harvested yield (350 kg/ha) it remained far from farmers' expectation. Therefore, one gosa is to assess in the study zones all kinds of agro-industrial by-products available as feedstuff sources for fish. This led us to consider palm oil and rubber industries and to cooperate with Soguipah (Société Guinéenne de

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Figure 1. Map showing the position of Forest Region which is one of the four natural regions of Guinea Republic having common border with three countries Sierra Leone, Liberia and Ivory Coast.

Palmier à huile et d'Hévéa), a national company that owns those two agro industries.

This paper concerns only Palm Kernel meal (PKM) bioconversion, a natural process used to transform this by-product to make it usable for feeding fish. Its composition in terms of nutriments will be also presented. A brief explanation on the sequence, which has driven us to discover the insect *H. illucens*, also known as Black soldier (BS), will be given. The first empirical culture method of BS larvae on PKM is hereby presented as well as the results on the first use of *H. illucens* larvae as feedstuff in tilapia culture.

MATERIALS AND METHODS

Palm oil industry by-products

The palm tree (*Elaeis guineensis*, Jacq.) is native of tropical West Africa. The fruits grow in bunches and consist essentially of a soft outer skin, which is reddish when it becomes ripe (Figure 2). The palm fruit produces two types of oils: the first category of oil named crude palm oil (CPO), red in colour, is extracted from the fibrous external layer, the mesocarp of the fruit. CPO is usually used as cooking oil. Inside the mesocarp there is a hard nut (endocarp) also known as the shell which encloses a kernel, itself containing a second type of oil, the palm kernel oil (PKO). The PKO contains essentially saturated fatty acids (Anonyme, 2001): lauric acid (C12: 48%), myristic acid (C14: 16%) and oleic acid (C18: 15%). It is used as basic ingredients for margarine production, confectionery, animal feeds and cosmetics such as soaps, creams.

To process PKO, the first step consists of breaking the hard shell (Akubuo and Eje, 2002) and separating from the kernel. The Palm Kernel Meal (PKM) is the solid part remaining from palm kernel after the extraction of PKO by a mechanical screw pressing. The palm kernel meal, also known as palm kernel expeller (PKE) or Palm Kernel Cake (PKC), has high protein (18-20% of crude proteins) and lipid contents (12 - 20% of crude fats). This variability of crude fats concentration is related to the technical extraction process mechanical only or combined with solvent used (Chin, 2002). In our case study, PKO were extracted by mechanical screw pressing only. Therefore nutrients level remaining in PKM is usually higher (18% of crude proteins and 20% of crude fats). PKM is rich in arginine, leucine and cysteine but deficient in lysine (Pérez, 1997) which may be increased by fermentation (Iluyemi et al., 2006) or supplementation with other lysine-rich sources, such as molasse or vegetable oil.

Every year, Soguipah oil factory have produced nearly 1000 metrics tons of PKM. A very few quantity have been tried with monogastric animals, such as swine, poultry and fish. The uses of palm kernel meal have been tried with the Nile tilapia as well as with juveniles of Labeo senegalensis, but lower values results of PKM digestibility has been recorded (Omoregie, 2001). The low digestibility of the nutrients from PKM has been found and explained by the high levels of non-starch polysaccharides (NSP) in the cell wall materials (Düsterhöft, 1993). The NSP is known as an antinutritional factor impairing the digestibility of nutrients contained in PKM (Choct, 2001). Only ruminants may use PKM with a greater efficiency because of the action from microorganisms (bacteria and fungus) and their enzymes breaking down the cellulose walls of the cells (Lim et al., 2001). A few quantities of PKM have been tried locally with ruminants (sheep or cattle) but farmers preferred to feed extensively with green grass. Overall, a large quantity of PKM remains unused. Among other agro-industrial by-products, consider-



Figure 2. Palm Kernel Meal (PKM): its origin and its composition in nutrients.

ing the percentage of proteins in the dried mater and the price of PKM, the cost effectiveness of proteins remains cheapest in PKM (Shayne, 2000).

Attempt to use PKM through fermentation process

As a consequence of what we observed in ruminants and through many articles (Düsterhöft, 1993; O'Mara et al., 1999; Choct, 2001), micro-organisms through their complex enzymes in the digestive track could play a key role in the biodegradation of PKM. From this point, an attempt of PKM fermentation was attempted by simply mixing the PKM with rumen contents and water. After two weeks of culture, fermented PKM (mixed with rice bran) was used to feed *Oreochromis*. However, the fermented PKM, blended with rice bran was not appealing to tilapia. The unused fermented PKM, because of the smell, are stocked at the backyard of the station. After two weeks, we have observed around the area, many chickens and birds feeding from it. Surprisingly we discovered that inside the containers, thousands of maggots have developed within fermented PKM. The maggots were larvae from the insect *Hermetia illucens*. The tilapia enjoyed the live maggots feed.

Hermetia illucens (Linnaeus) (Stratiomyidae - Diptera)

At first sight, H. illucens (also known as Black Soldier fly) larvae were not identified as such. They were found, as mentioned above, among fermented PKM forgotten at the backyard of the station. We initially mistook BS larvae for common blow flies maggots. And when we saw sometimes Black Soldier flies (H. illucens) alone, we were again mistaken by taking them as wasps. However, the larvae morphology and their size and weight (24 mm long, 8 mm wide and 240 mg weight) were so unlike fly larvae. Secondly, the duration of metamorphosis observed on those larvae (from eggs up to pupa stage) was much longer. The remarkable difference also concerned the pupa morphology. Because of this, we decided to enclose 50 pupae (at the black stage) in a 4 L transparent plastic container with a cover where we made small holes for air to pass through. After two to three weeks, we found in the plastic container a few insects black in colour hatched leaving empty black soughs. From that time it was clear that the larvae appeared among the fermented PKM

were not from the ordinary flies (blow or green flies). The specimens of insects and larvae were preserved in 75% ethanol. The identification was the book "Immature insects" (Stehr, 1991; Chu and Cutkomp, 1992). It was clear that the larvae we have found, are the members of Stratiomyidae family and Diptera Order. Further search on the Internet led to know more about the insect. *H. illucens* has been recognized by many authors and scientists as one of the most useful species (Warburton et al., 2001). Recent studies (Sheppard, 1992; Newton et al., 1995) have shown that *H. illucens*, in larvae stage, have been used to reduce chicken and pig waste to a non-polluting residue in a matter of days under ideal conditions. *Hermetia* larvae biomass has been also used to feed fish and chicken.

In terms of biodiversity, *H. illucens* have been found in a large geographic range, around the world throughout warmer regions between latitudes 45° North and 45° South including the Australasian and Oceanic Regions like New Caledonia (Rageau, 1957; Cochereau, 1970,1974), Solomon Is, Vanuatu, Western Samoa, French Polynesia, etc.

Before making the decision to work on *H. illucens*, some previous investigations are needed to ensure that the insect species are not dangerous or have undesired negative side effects. According to many investigations, BS is well known as a useful insect (Sheppard, 1992; Newton et al., 1995; Leclercq, 1997). The short duration of the adult life may explain the character of the non pest aspect of Hermetia fly. However, one myasis case has been mentioned in Costa Rica (Calderon-Arguedas et al., 2005). *Hermetia* larvae is also used, in medical jurisprudence (Turchetto et al., 2001), as a forensic tests to esti-mate the period of a crime detection. Considering many advantages related to the BS role in biodegradation, it was reasonable for us to continue to carry on working with *H. illucens*.

Production of BS larvae

In terms of systematic classification, *H. illucens* is known with precision, but by contrast, their biology, biotope and habitat use as well as their breeding ecology, remain poorly known under tropical climate conditions. The production techniques applied during the year 2001 were based purely on empirical observations. The first mass cultures were done in rectangular iron tanks, dimensioned as follow: $1.5 \times 3 \times 0.4$ m deep (Figure 3A). The whole structures were kept 0.5 m above ground. Eight tanks were used for the culture. The culture medium (Palm Kernel Meal, mixed with water: 1 kg PKM for 2 L of water) was placed on the culture tank bottom. The layer thickness of the medium must be 3 to 4 cm (Figure 3B). The culture tank as dimensioned above may receive 80 kg of dried PKM.

The optimum time of culture was not yet known at that time. The harvests have been executed according to the estimation at a rough guess on the quantity of larvae appearing in the medium. The larvae were separated from the medium by filtering and cleaning with water (Figures 3C and 3D). Among the data recorded, we have considered the amount of input PKM, the starting date, the harvest date and the biomass of larvae collected. When some time the cultures were destroyed by rain flood or by predation from wild animals, those data were not considered. The results showed that the conversion rate (the ratio between the quantity of PKM input and the biomass of larvae harvested) was very variable with an average = 5.8 and a standard deviation 3.2.

Through the graphic interpretation (Figure 4) by placing conversion rate (PKM kg/Biomass larvae kg) on the Y-axis and duration of culture time (the numbers of days between the started date - and the harvested date) on X axis, we observed an inverse correlation between the two parameters (r = negative). It means when the culture duration is longer, the conversion rate is low. Otherwise the conversion from PKM to larvae biomass was more efficient. According to this result, it is clear the optimal culture time is more than three weeks. From then on we fixed 4 weeks as the optimal time for harvest. Tanks must be covered by chicken wire in order to protect the culture from rats, lizards, birds, or other animals. The tanks must be also roofed otherwise the whole culture may be flooded by rain.

Application of *Hermetia illucens* larvae as feedstuff in tilapia culture

This is not the first time *Hermetia* larvae has been used for feeding fish. Bondari and Sheppard (1987) have tried to use BS larvae since 1981 to feed catfish *Ictalurus puntatus* and *Oreochromis aureus*. The authors have found that growth was satisfied of the two species feeding with BS. Chopping of BS larvae improved weight gain and efficiency of the utilization.

RESULTS AND DISCUSSION

Since June, 2002, production of BS larvae in Forest Region has become a routine. Production about 30 kg of fresh BS larvae have been obtained weekly from the culture. Direct feeding with live BS larvae to *tilapia* in pond has confirmed that the fishes appreciated greatly this new feedstuff (Figure 3E). From that stage we have proposed for the first time to design a simple formula based on BS larvae and rice bran (70% of rice bran and 30% BS larvae) the only ingredients available in the region. The proportion of each ingredient is based on their dried matters (6% of water in rice bran and 70% of water in SB larvae).

Four hundred males Tilapia about 20 g of body weight (*Oreochromis niloticus*) were placed in pond (20 x 20 x 1.5 m). The feeding rate was started from 7.5% of biomass and ended at 4% at the sixth month before harvest.

The mortality was relatively high at the beginning of the experimentation (3% during the two first months) and stabilized around 1% until harvest. At the end of the culture the individual daily growth rate was at the average of 1.8 g/day was recorded. It means the growth rate is 3.5 times faster than culture feeding with rice bran alone (0.52 g/day).

This first result has opened a new desire to promote aquaculture in the region. December, 2003 marked the end of the project, but the culture of BS continued as well as tilapia culture. Now, replication of the experiments have confirmed the process. The value of growth rate from 1.5 to 2 g/day, resulted from 6 months of culture of tilapia male (density of one per m²), from 20 g initial weigh to 300 g final weigh. The formulation of 30% of BS larvae and 70% of rice bran, based on dried mater, has become the standard in the studied zone.

- A Fermentation phase (dried PKM + water; 1:2)
- Biodegradation phase by *Hermetia* larvae (four weeks)
- C. Harvest of lawae biomass
- Larvae biomass ready for use as feedstuff for fishes
- E. Tilapia culture using Hermetia larvae





- Formulation based on dried matter: 30% maggots + 70% rice bran
- Daily ration 5% of biomass
- Growth rate ~ 1.5 g/day
- Average weight 350 to 400 g after six months of culture.

Figure 3. Bioconversion process is started from fermentation of PKM (with water). After one week, the smell of PKM fermented attracts the insect *Hermetia illucens* to come and lay their eggs on it. *Hermetia* larvae biodegrades the PKM and develops within the medium (PKM). Biomass of larvae obtained is used as foodstuffs for tilapia culture.



Correlation between conversion rate and number of days of culture

Figure 4. Relationship between the conversion rate and the duration of culture (number of days).

Conclusion and recommendations

The finding of this bioconversion process on PKM by black soldier larvae has opened a new hope for tilapia rearing in the rural areas where agricultural by-products are often available. This experience can be transferred into others regions and countries in West Africa where PKM are abundant. The time limit of our project did not permit us to accomplish the research on BS life cycle. So far the study referred to data from literatures published in the United Stated and Australia. This lack of knowledge concerning tropical zones need to be filled.

PKM is not the only by-product that can be used as medium for biodegradation. Future attempts should include other organic by-products or domestic wastes (Newton et al., 1995). Research technique must focus on mass production of BS larvae in order to respond to the real scale of fishmeal replacement need. For a large extension program, we must keep in mind and concentrate effort on rural conditions in order to innovate the low key technology that do not require heavy cost investments. Once the mass production of BS larvae is achieved, research must continue on nutrition aspects in order to perform the formulation for fish or for animal feed in order to reach a better profitability for rural farmer.

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Valorization of Palm Kernel Meal via Bioconversion: Indonesia's initiative to address aquafeeds shortage

Saurin Hem, Melta Rini, Chumaidi, Maskur, Ahmad Hadadi, Supriyadi, Ediwarman, **Michel Larue and Laurent Pouyaud**

This article outlines the initiative of Indonesia to convert "waste to wealth" through the natural process of bioconversion to produce aquafeeds from palm kernel meal for the country's rural aquaculture. This is based on a poster paper presented by the authors during the International Conference on Oil Palm and Environment (ICOPE), 15-16 November 2007, Bali, Indonesia.

ndonesia's demand for aqua feeds, specifically the commercial fish pellets has increased due to the expansion of its rural aquaculture sub-sector. However, the price of aquafeeds or commercial pellets for aquaculture nowadays has become unaffordable to many small-scale fish farmers due to the ever increasing price of fishmeal, which the country imports from Peru, Chile, and other South American countries. The price of fishmeal also continues to soar not only because of its high demand worldwide but also because of the stagnation of fish production from the natural resource. Indonesia spends about USD 200 M per year for the imported fishmeal for its aquaculture industry. If such situation continues, the development of the country's aquaculture will be hampered. Fearing further decline of fish production from aquaculture while boosting the rural economic sector to address the country's food security concern, Indonesia has tried various alternatives to produce aquafeeds using locally available ingredients.

Many research efforts related to the production of alternatives to fish meal have already progressed in many countries not only in Asia but also in the Americas. The use of insects as source of protein in fish diets has already been successfully tried. In China for example, the nutritive value of insects as feeds for cultured fish has already been recognized where studies have demonstrated that insect-based diets are cheaper alternatives to fish meal. The most popular insect used in this particular case is the Black Soldier (BS) fly, Hermetia illucens (Stratiomyidae, Diptera).

BS fly is a non-pest tropical and warm-temperate insect that has been found useful for managing large concentrations of bio-solids as well as other by-products and wastes. A cosmopolitan species that is widespread in Indonesia, the larvae (maggots) of the BS fly at first sight might look like those of the blue fly or house fly. However, there is a huge difference since the latter species are true pests, while the BS fly is rather a "flower species" and not a pest at all. Its



usefulness as source of protein for fish culture has been recognized since the 1950s by many researchers from the USA. Many research studies on the larvae of Hermetia illucens have also been conducted in some Southeast Asian countries and expanded in Indonesia, after a huge population of the BS fly was spotted in Sukabumi and Depok Provinces of West Java.

Bioconversion of Palm Kernel Meal

Indonesia is the second world producer of palm oil after Malaysia. Aside from palm oil, the industry yields huge amounts of palm kernel meal (PKM or Bungkil). PKM is a by-product after palm oil has been extracted from the African Oil Palm (Elaeis guineensis), which was introduced in Sumatra in the early 1900s. It was reported that in 2006, Indonesia produced about 2 million tons of PKM of which only one-half was exported. Since so much PKM is available and sometimes considered as wastes, the country's fisheries sector is conducting a bioconversion research program which aims to address two-tailed concerns: reducing the need for imported fishmeal for its aquaculture industry; and value-adding a local resource, the locally-produced PKM. However, proteins and fats locked in the PKM can not be used directly by fishes (Hem, et. al. 2008), but since the enzyme from maggots, e.g. larvae of





H. illucens could be used as fish feeds, this leads to maggot biomass production through bioconversion.

Bioconversion is a natural process which consists of the transfer of nutrients via biodegradation using the larvae of an insect. It has been considered the cleanest, most efficient and most economical way to recycle waste products. Since bioconversion does not require electricity, chemicals not even water, it does not produce any greenhouse gases, and the process does not require any imported technology. The agent chosen for the bioconversion process of the PKM in this Indonesian initiative is the BS fly.

As previously reported, BS fly has been found effective in reducing the mass of solid wastes. Fish feeding experiments and analysis also indicated that dried BS fly prepupae grown in selected solid wastes have the nutritive value required in cultured fishes. Since BS fly is capable of converting residual protein in solid wastes and other nutrients into biomass, it could produce high quality protein feedstuff. Some studies have also proved that pollution reduction could be one of the returns for good bioconversion management.

While research studies conducted in some countries made use of fungus and insects such as silkworm, housefly, etc. for the bioconversion of PKM to produce fish feeds, a key step in the bioconversion process in Indonesia is the elucidation of the life cycle of the BS fly, *Hermetia illucens*, with particular emphasis on its reproductive biology (breeding behavior, reproductive cycles, etc.). As demonstrated in previously reported research works, the resulting biomass of larvae (42% crude protein and 30% crude fat) acts as a viable alternative source of animal protein for sustaining the development of aquaculture.

Recently developed in Indonesia, the PKM bioconversion program aims to promote an in-depth understanding of the bioconversion process and at simplifying the production process of the maggot feeds or "magfeeds" so as to promote its implementation in the rural context. The initial application in Indonesia of the bioconversion technology at a small-scale level (1 mt of magfeeds per month) has been validated in 2006-2007 at its Aquaculture Development Center (Balai Budidaya Air Tawar or BBAT) in Jambi Province.

Way Forward

The country's pilot PKM bioconversion project planned in 2008 will aim to produce a maggot biomass of 10-15 mt/month with direct application to aquaculture. The bioconversion of PKM into "magfeeds", a natural process dubbed "from waste to wealth", is a promising research topic. With the objectives of addressing local needs with local resources, it could also contribute to fishmeal replacement in a broader, worldwide context, since the bioconversion agent is locally available. Furthermore, "the capacity of a country to produce local resources that substitute imported products represents a strong criterion of sustained economic growth."

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A good example of successful bioconversion



Developent of the bioconversion process near oil palm plantations will bring better conservation of the tropical forest, the natural habitat of Hermetia illucens.

Oil palm is the predominant source of vegetable oil in the world. Growing population could bring a doubling of demand in 10 years' time. Its production is accused by environmentalists of steadily destroying the primary forests, yet it has become indispensable for making food products (such as biscuits, crisps, margarine and vegetable butter or soups). The harmful impacts of the monospecific crop on the biodiversity and deforestation are well known, but the difficult valorization of the plant wastes resulting from this agro-industry is less well realized. Palm kernel meal (PKM), a fibrous material obtained after pressing of palm fruit, is still difficult to recycle. An IRD team has been studying the potential of oil palm production by-products for several years and has recently developed a highly promising bioconversion process. Observations on the interest the dipteran *Hermetia illucens* or black soldier fly, a common species in the tropics, shows for PKM prompted the research team to investigate the biology of this insect. Previous work conducted in the Republic of Guinea had shown the

larvae of this insect to be capable of extracting the nutrients (proteins and lipids) from the plant fibre residues. Research has been proceeding in Indonesia since 2005 where the team has been aiming to develop a bioconversion process that applies these insects' remarkable digestive abilities. The country has recently become the world's top producer of palm oil and this production generates 2.3 million tonnes of PKM. In the Indonesian archipelago, freshwater fish farming is a flourishing traditional economic activity which requires \$200 million worth of fish meal imports every year. The combination of these two factors gave the idea of developing large-scale production of Hermetia larvae as an alternative to fish meal which, with the surge in prices linked to the steady depletion of natural stocks, is an increasing burden on the country's trade balance. The IRD is soon to set up a pilot production unit in Sumatra near an oil plant that produces 50 tonnes of PKM per day and this will allow the production of an abundant quantity of larvae. The process is based on a fermentation system inspired by ruminant

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ntensive cultivation of oil palm is strongly criticized for the destruction of primary forest it causes. In addition to its effects of deforestation, this activity produces environmentally harmful organic residues. Indonesia, which recently became the world's leading producer of palm oil, generates an annual 2.3 million tonnes of this by-product, palm kernel meal. However, a means of beneficially using these solid plant wastes is emerging thanks to a process being developed by IRD researchers and their Indonesian partners¹. The technique draws on the remarkable digestive abilities of maggots (larvae) of a dipteran insect, the "black soldier fly", which produces a great quantity of biomass rich in protein. This substance provides a cheap feedstuff which substitutes for the fish meal usually employed by the region's aquaculture farms. The waste residues the larvae produce also serve as an excellent green fertilizer. When applied to leguminous crops, it can result in yields three to four times higher than a plot that has not been so treated.

For further information

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digestion combined with the enzymatic action of the Hermetia larvae. It consists of setting out tanks containing a mixture of PKM and water on the edges of the tropical forest near Hevea (rubber) plantations. The female flies are attracted by the odour of fermenting palm kernels and come to them to lay their eggs. Once hatched the larvae develop, feeding on this pre-degraded substrate during the fermentation phase. This method, whose efficiency has been improved over recent years, can currently supply one tonne of larvae from 2.5 tonnes of palm kernel in three weeks. When the larvae stop feeding, they are harvested and then mixed with rice bran, another agricultural by-product easily available in Indonesia. The feed thus obtained provides the essential nutrients for the fish species bred in aquaculture such as tilapia or panga. But the virtuous circle the scientific teams has devised does not stop there. More recently, observations and measurements were made on the fertilizing properties of the excrement produced by the larvae. The first tests conducted on the asparagus bean (Vigna unguiculata sesquipedalis), a leguminous plant commonly grown in Indonesia for its edible pods and seeds, have proved that the excrement constitutes an excellent green fertilizer that makes possible yields at least four times greater than those of non-fertilized crops. This virtuous circle system, which takes its inspiration from the principles of functional ecology, proves that nature can provide a service for humans for less cost when it is harnessed in a reasoned way.

The researchers hope that by encouraging local communities to use this bioconversion process, the ecological impact of oil palm cultivation will be restricted while favouring the conservation of the forest ecosystems in Indonesia. The tropical forest is the natural habitat of *Hermetia illucens*, therefore the bioconversion process devised can work only if this ecosystem is preserved. This is an argument that could give agro-industrial companies incentive to leave small islands of primary forest between the oil palm plantations.

Grégory Fléchet - DIC

Translation - Nicholas Flay

1. This research was conducted as part of the FishDiva programme in cooperation with the Indonesian Ministry of Fisheries and Maritime Affairs.

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IRD /



Left: Black soldier maggots resulting from the bioconversion process. Right: green fertilizer obtained from the digestive wastes of these larvae.

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From Palm Kernel Meal (PKM) to Minilarvae Biomass, via a natural process : Bioconversion

1. Context

Aqua feeds or (commercial pellets for aquaculture fish) are becoming increasingly expensive worldwide. This situation (5-6 millions/year). The situation is proportionally more critical in the developing countries. Research focusing on the new resources of feed so called fishmeal replacement become a real need today. Bioconversion of PKM research program is part of this topic.



2. Opportunity

Indonesia is the first world producer of palm oil. Aside of palm oil, this industry yields huge amounts of a by-product, the palm kernel meal (PKM or Bungkil; about 2 million tons in 2006), of which half is exported. The bioconversion research program precisely aims at valorising the locally produced PKM so as to produce animal proteins that can contribute solving the issue of alternative protein sources to imported fish meals.

Bioconversion is a Bio-process using natural enzymes (proteases and lipidases) secreted and excreted from insect larvae (*Hermetia illucens*, Stratiomyidae, Diptera) aka Black soldier fly.



PKM by product palm oil agro-industry (image taken from factory

3. Bioconversion, a natural process and applications

The bioconversion process consists in the transfer of nutriments of PKM via biodegradation by larvae of an insect, Hermetia illucens (Stratiomyidae, Diptera) also known as Black Soldier (BS) fly, a cosmopolitan species that is widespread around the globe. A key step in this process is elucidating the life cycle of Hermetia illucens, with particular emphasis on its reproductive biology (breeding behaviour, reproductive cycles, etc.). The resulting biomass of larvae (48-52% crude protein) may act as a viable alternative source of animal protein for sustaining the development of aquaculture.

Recently developed in Indonesia, the bioconversion program may produce two kind of products : (1) small size larvae named "Minilarvae" with body size from 1.5 to 4mm large, destined to be used as live food for ornamental fish and (2) pre-pupa large size larvae or "Big Maggot (BigMag) from 5 to 7mm of body diameter. "BigMag" may be use as live food for fish and poultry or convert into dry form to be incorporated in pellet formulation as an alternative of fishmeal.



5. Actual stage and future

Pilote scale of Minilarvae production is planned to run in October 2009 to generate monthly 400-500 kg of Minilarvae biomasses . For the mass production of "BigMag", an other pilot project is actually running, implicating a group of farmers with an aim to produce a biomass of 4-5 tons per month of BigMag to apply directly to rural aquaculture in the same village. This pilot program, occurred in Jambi Province (Sumatra) will take one year period of experience. An assessment of this show case will be analyzed on November 2009. The bigger scale application will occur

6. Concept and Conclusion

The bioconversion of PKM into "Maggot", a process dubbed "from waste to wealth", is a promising research topic, with the objectives of addressing local needs with local resources, but also of contributing to fish meal replacement in a broader, worldwide

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after the lessons learned from the attempt during this year. The bioconversion of PKM into "Maggot", a process dubbed "from waste to wealth", is a promising research topic, with the objectives of addressing local needs with local resources, but also of contributing to fish meal replacement in a broader, worldwide context, since the bioconversion agent is cosmopolitan. Furthermore, "the capacity of a country to produce local resources that substitute imported products represents a strong criterion of sustained economic growth".

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Valorisation 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

1. Context

Aqua feeds or (commercial pellets for aquaculture fish) are becoming increasingly expensive worldwide. This situation arises from an increasing demand over fishmeal and fish oil, while captures of wild fish have attained a plateau. Fishmeal imports in Indonesia nowadays amount to circa 200 million US per year, and are expected to soar if no alternative to fish meal proteins were found. The situation is proportionally more critical in the rural sector, where aquaculture has played an important part since several generations and where production is mainly or exclusively destined to local markets.



2. Opportunity

Indonesia is the first world producer of palm oil. Aside of palm oil, this industry yields huge amounts of a byproduct, the palm kernel meal (PKM or Bungkil; about 2 million tons in 2006), of which half is exported. The bioconversion research program precisely aims at valorising the locally produced PKM so as to produce animal proteins that can contribute solving the issue of alternative protein sources to imported fish meals.

Lauric acid (C12) ~ 48 2. Crude Palm Oil (CPO)

Bioconversion is a Bio-process using natural enzymes (proteases and lipidases) secreted and excreted from insect larvae (Hermetia illucens, Stratiomyidae, Diptera) aka Black soldier fly.

3. Bioconversion, a natural process and applications

The bioconversion process consists in the transfer of nutriments of PKM via biodegradation by larvae of an insect, Hermetia illucens (Stratiomyidae, Diptera), also known as Black Soldier (BS) fly, a cosmopolitan species that is widespread in Indonesia. A key step in this process is elucidating the life cycle of Hermetia illucens, with particular emphasis on its reproductive biology (breeding behaviour, reproductive cycles, etc.). The resulting biomass of larvae (42% crude protein and 30% crude fat) may act as a viable alternative source of animal protein for sustaining the development of aquaculture. Recently developed in Indonesia,

the bioconversion program aims at an in-depth understanding of the bioconversion process and at simplifying the production technology so as to promote its implementation in a rural context. The application of this technology at a small scale (1 metric ton per month) has been validated in 2006 - 2007 at the Aquaculture Research Station of Jambi Province (BBAT). In 2008, a pilot project will aim to produce a maggot biomass of 10-15 tons per month with direct application to rural aquaculture.



4. Nutritive values

amino acids essential	Eich	Eich		н	ermetia lar	vae meal (HLM)								
	meal (Chile)	meal (Peru)	Average	HLM HLM (IRD-BRKP Indonesia) 1977)		HLM (C. Sheppard- Swinerej ect.)	Average		Soybean	Rice bran	Maize	РКМ	Coconut	Soybear meal
Arginine	5.9	5.7	5.80	1.6	2.2	1.7	1.8	1	4.6	6.2	4.6	13.9	11.0	7.4
Histidine	2.6	2.3	2.45	1.1	1.9	1.0	1.3		2.3	2.3	2.9	2.5	2.1	2.4
Leucine	7.6	7.5	7.55	2.4	3.5	2.6	2.8		6.0	7.3	13.1	6.4	6.0	7.8
Lysine	8.0	7.5	7.75	1.9	3.4	2.2	2.5		3.6	4.1	2.4	3.7	2.5	6.1
Methionine	3.1	3.0	3.05	1.0	0.9	0.8	0.9		1.8	1.8	0.6	2.7	1.0	1.4
Phenylalanine	4.3	4.2	4.25	1.4	2.2	1.5	1.7		4.0	4.5	4.9	3.6	4.1	5.5
Thréonine	4.3	4.1	4.20	1.5	0.6	1.4	1.2		4.2	3.4	3.6	3.5	3.0	3.8
Tryptophan	1.2	1.1	1.15		0.2	0.6	0.4			1.6	0.6	2.8		1.3
Valine	5.5	5.2	5.35	2.0	3.4	2.2	2.5		4.7	5.5	4.2	5.7	4.7	5.2
				- Alerent	6.63.7									
Crude proteins	72.6	1.1	71.6	44.0	42.0	42.0	42.7		26.4	10.6	10.6	18	22.7	44.0
Crude fats	1.1	1.1		29.35	35	35	33.1		19	18.9	1.9	32	10.5	8.1



5. Concept and Conclusion

The bioconversion of PKM into "Maggot", a process dubbed "from waste to wealth", is a promising research topic, with the objectives of addressing local needs with local resources, but also of contributing to fish meal

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economic growth".



replacement in a broader, worldwide context, since the bioconversion agent is

cosmopolitan. Furthermore, "the capacity of a country to produce local resources

that substitute imported products represents a strong criterion of sustained



meal by steaming, drying and grinding for preservation. 6. Selected pupas for restocking purpose

Soybean x HLM x1,5

15 - 16 Nove

Up to 50 tons of PKM are evacuated each day in this factory near Jambi (Sumatra)

UK fishmeal prices 2003 to 2006 (Source FIN Fishmeal Information Network) http://www.gafta.com

mber 2007

Bali, Indonesia

Report on the establishment of "Bioconversion" pilot project at desa Singkut 1

(Kebupatan Sarolangun – Jambi - Sumatra)

By Saurin HEM

From 14th of March – to 27th of March 2009

1. Historical reminder

The bioconversion program has reached at 2009 a decisive step. A significant financial support from the government through BRKP and Dinas DKP of Jambi province has an objective to conduct the pilot program of bioconversion consisting on putting in practice the principle of the bioconversion as a service for rural people that has faced with aquafeed issues with the fishmeal shortage. Sarolangun district was one of those cases where aquaculture activity was closing down. The village Singkut 1, part of the district, has beneficiated this first example support.

The meeting held on the 4th of March at Novotel Jambi, has discussed on the design and method used to coordinate the roles and actions of each party involved (BRKP, IRD and the DGA/DKP Dinas of Jambi). All parties are agreed on the conception of Pilot Project. The objective of the project must go beyond the technical process of producing maggot. The Pilot project must act as a demonstration of the whole transformation chain from maggot up to fish production as the final focus. The Pilot project must demonstrate that **maggot as a new source of animal proteins to replace fishmeal and to provide low cost aquaculture**. Therefore the pilot project must cover the three steps of the process started (1) from maggot culture followed by (2) the pellets fabrication based on maggot biomass. The project will continue to follow up until (3) fish culture and harvesting. Each step must include the economic consideration taking in account all costs involved (investment, infrastructure, running cost,...). On the other word, the frame of the project may be presented as a business linking chain of three specific unit groups of operators:

- The first group of operators, or Group A, will be in charged on maggot biomass production.
- the second group of operators, Group B, will be in charged on aquafeed pellet production. The group B will buy the whole biomass of maggot produced from group A, to be used as the main ingredient inputted into the pellets composition, instead of fishmeal. The pellet produced will be free from fishmeal.
- The third group, Group C is composed by fish farmers practicing aquaculture in ponds. They will buy pellets from group B for growth out their fish in respective ponds.

Singkut 1 village was focus to play as pioneer in this program. The families involved in the operation are identified and decided by farmers association them self.

The agreement has also decided on the duties and responsibilities of each party as shown as follow:

- 1. The duty under IRD responsibility is to set up 2 (two) maggot culture sites (two families among the 5) and to build one storehouse for stocking Palm Kernel Meal.
- 2. Dinas Perikanan of the Jambi province is in charge on the construction of three other maggot culture sites (three families). The duty of Dinas will provide also the PKM (72 tons) and will insure it transportation from the factory up to storehouse at Singkut 1.
- 3. The duty part of BRKP is to ensure the running costs between three groups of operators: maggot producers (5 families), aquafeed maker (1 family) and fish-farmers (12 families). The running cost concerns firstly the maggot purchased by the aquafeed maker, and secondly it concerns the fingerling cost for the growth out operation in the 12 families.

Another point discussed during the meeting has focused on the technical design related to the maggot culture backs (3,5 m x 7 m with 0,60 m high), covered by zinc roofing, which is planned to establish 4 in each site; the PKM storehouse dimension (5m x 5 m x 3 m high) for stocking 25 - 30 tons of Palm Kernel Meal). The construction of a pellets unit-workshop at Singkut 1 was designed under responsibility of Dinas of Jambi province.

2. Mission activities – field operations

Following the schedule decided in the pilot program, the period from middle of March to the end of April, will devote on the construction activities to setting up all infrastructures and facilities planned at Singkut 1.

The IRD mission reported here concerns the construction of the two (of five planned) maggot culture sites, according to the design and dimension decided (see figures). The two families involved in maggot production placed under charge of IRD, are : family Basuki (site $n^{\circ}1$) and family Omon (site $n^{\circ}3$). The storehouse of PKM will be installed in the family Dadang located in the central of the village (See GPS map).

Pasar Singkut finally is an active agglomeration where all construction materials (iron bars for reinforced concrete, cement, send,...) are all available in place there. Three local contractors are found in place among the farmers group. All are professional in concrete masonry work and may understand without difficulty all designs and construction dimensions prepared by IRD.

Without theodolite instrument to accurate the positioning of the construction in a vast areas, we have had recourse to the principle of Pythagorean theorem. This is a simple solution interesting to transfer to rural areas when they will need to accurate measurements in case of large construction surface.

Most of the soil textures in Singkut are alluvial origin (glay, send,...) that are loose at the surface. All heavy constructions need to insure by a solid foundation (case of Basuki and Omon). This context has taken more time and cost to reinforce the foundation of the infrastructure;

In Basuki case (site $n^{\circ}1$), the construction of the culture back has followed the plan deseigned (20 m long x 8 m large) decided during the 4th of Mars meeting. But in the Pak Emone case, the area designated for the construction is narrow. The disposition of the 4 culture backs needs to be changed. The disposal areas dimension is 30 m long x 6m large. However the surface of each culture back remains the same following the model decided (7 m x 3,5 m x 0,6 m). The whole structure is covered by corrugated zinc roofing panels.

Two problems have been faced during the construction:

- The raining day where all work must cancel. This has caused some delay in the schedule
- The new bridge recently build at the entrance of the village was not yet solid to allow heavy truck to cross over. Thereby all the construction materials (iron bar, cement, send, block of stone, ..) transported by heavy trucks (8 to 10 tons) must be dropped before the bridge and retransferred by small quantities up to the construction sites. This has caused the increasing cost in the construction as well as disturbing the time schedule.

Insect house for restocking:

The restocking program has planned in the schedule but the chapter doesn't have designed and discussed in detail. IRD has take this part under responsibility.

Four (4) stocking boxes will be setting up within each culture site. Every week after harvesting the maggot from the culture tank, about 5% of larvae produced, especially the pupae stage (the last stage of development) will be inputted in the insect house for restocking. All pupa are ready to hatch and fly into the adult stage. Nearby the insect house, the areas around it will be prepared to plant the daisy flowers *Sphagneticola sp.* to create an habitat for the adult insects.

Incompatibility with the spray of two pesticides "Round'up from Monsanto and Gramoxon" - an alternative solution, a biological control by planting the daisy flowers Spharneticola sp

Frequently, the farmer used to spray the herbicide under the rubber plantation against the development of the adventices that would compete and disturb the culture system (rubber). The farmers usually use two herbicides, pesticides: the first the famous "Roundup from Monsanto" - glyphosate (N-(phosphonométhyl)glycine, $C_3H_8NO_5P$)- (<u>http://biogassendi.ifrance.com/editobiofr.htm</u>) and the last but not the less is the "Gramoxon" (PARAQUAT CHLORIDE or 1.1-DIMETHYL 4.4'-BIPYRIDIUM DICHLORIDE, $C_{12}H_{14}CL_2N_2$) both are well

known as a pesticides deeply damaging environment (water and soil) and dangerous for the users, farmers themselves. <u>http://www.find-health-articles.com/rec_pub_897259-a-case-fatal-intoxication-gramoxon-author-s-transl.htm</u>

These practices are not compatibles with the maggot production program while insect population *Hermetia* illucens is the background of maggot production. Therefore, the farmers must accept to stop using those pesticides in the areas near by the culture site.

The questions frequently asked are:

- how to prevent the adventices, bad grass, from growing which compete and disturb the agricultural system ?
- and what could be the alternative solution ?

According to our experiences in Depok, using the daisy flowers *Sphagneticola sp.* as the habitat of insect *Hermetia illucens* in the enclosed culture has shown many advantages. *Sphagneticola* colonies will compete and prevent the

adventices from growing, by obstructing the sun light (reducing the possibility of photosynthesis) by the thickness of their colonies over the soil areas. If this biological control of adventice pest could play this role, this new action may



contribute the farmers from stop using the two pesticides mentioned above. The action will reduce the bad impact on the environment and will help the farmers from spending money every month fbuying the two pesticides (75.000 Rp to 100.000 Rp for 2 ha of culture). This biological control by *Sphagneticola* will overall contribute to save the environment (water and soil) and provide a better health care on the rural population. This experience will take one year before to make a conclusion after a clear objective analysis. A new research program may develop around this topic.

Restocking boxes

The natural population of *Hermetia* exist actually at Singkut1 but may not be able to ensure the high reproduction immediately. To obtain the high production started from the beginning, a restocking of pupa is needed. We have plan to transfer pupa from BBAT of Jambi.

We have planned to equip for each site 4 boxes for stocking pupa. Twenty (20) boxes will be fixed in the 5 sites at the rate of 4 boxes par site. Two to three kg of pupa will be inputted every week. The design of the restocking boxes is:





Conclusion

During the two weeks stay in place, the purpose of the mission is to explain to the local contractors the accurate of the design, that will be used as model for others constructions which are under Dinas program.

The whole infrastructure could not be finished during the mission (two week). It will be completed some time before middle of April. All construction materials are precisely estimated and all are paid and available from the suppliers at Pasar Singkut (Cement, zinc, woods...).

Wisnu, my assistant from BBAT Jambi has followed the construction during 7 days. He would like to continue. But he has his program duties at the BBAT to control. For this point, I present a special thank to Pak Supriyadi (the head of BBAT Songail Galam) who kindly allows Wisnu to accompany the mission during this stay at Singkut village by helping in different contact (suppliers, contractors, workers and the bank transactions). Wisnu has learned also many technical aspects during this starting period. Firda, another young assistant has also participated at the start of the program.

Agung Dwi is nexly graduated from the STP (Skola Tingi Perikanan at Pasar Minggu) a new staff recently recruited by the DGA and posted at Sarolangun. Agung has played a important role in this project. Agung has followed the program conscientiously every step of it progression. The instructions confided are well execute after my departure. He is a good field engineer who may conduct smartly all the fieldwork. He remains keep in touch with me every day (Jakarta-Singkut) and sends the pictures of the work progression every 3 days via mms.indosat.com using his hand phone camera.

Pak Spriyadi a staff Dinas at Sinkut has also a key role in term of mediator.

I appreciate Pak Urip (BRKP-Depok) and Vidodo both have contributed efficiently the work.

In spite of some troublesome (rain, new bridge, contractors,...) causing delay, the program will finish within the schedule planned.

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View A – Profile view (All figures scale references are in Metric System)



Image in the direction of view A (at the beginning of the construction, picture dated 17-03-2009)

Image in the direction view A (the construction on the way, picture dated 05-04-2009)









Image in the direction of view A (at the beginning of the construction, picture dated 17-03-2009)



Image in the direction view A (the construction on the way, picture dated 05-04-2009)

Construction of the storehouse of Palm Kernel Meal PKM (25 m² for a stocking capacity from 20 to 25 metric tons of PKM) Site N° 6 – Pak Dadang.





Front view and dimension - scale references are in Metric System



Image taken at the beginning of the construction, positioning of the future storehouse (picture dated 20-03-2009) Finishing the storehouse (picture dated 05-04-2009)

Site N° 2 – Pak Emore (Village Singkut 1 – District of Sarolangun – Jambi – Sumatra)



Site N° 4 – Pak Dadang (Village Singkut 1 – District of Sarolangun – Jambi – Sumatra)



Site N° 5 – Pak Umar (Village Singkut 1 – District of Sarolangun – Jambi – Sumatra)



Madagascar Mission - Expertise Report

by Saurin HEM (IRD/BRKP – Indonesia)



Mission made from 16^{th} to 30^{th} of October 2010

Madagascar Mission Report

by Saurin HEM

Proposition of a tryout on Fishmeal Replacement by Maggot-meal applied with shrimp culture

Context and historic

Since 2009, research program on Bioconversion (known as Maggot research Program in Indonesia), has been recognized and appreciated by WWF in Paris. During SHAD (Shrimp Aquaculture Dialogue) conference held in Jakarta on 11-12 of March 2010, organized by WWF, Jacques Slembrouck and I, we were invited. Surprisingly, Bioconversion (Maggot) program was well known as a good concept on fishmeal replacement. Through many discussions and exchanges among participants, in particularly with Dr Marc Le Groumellec, who is part of Shad members and also one of the founders of Aqualma Shrimp Industry in Madagascar, has expressed his wish to visit Depok research Centre just before his flight back to Madagascar. After the visit, Dr Le Groumellec has appreciated our work and has expressed his intention to introduce a new approach Aqualma policy by proposing a tryout research program to substitute Fishmeal by Maggot-meal in the shrimp feed formulation.

AQUALMA, part of UNIMA Group, is a multinational created in 1994 funded by French and Madagascar capital, specialized on Shrimp Farming Industry (*Penaeus monodon*) installed on the west coast of Madagascar (precisely at Mahajamba bay see map attached). Today Aqualma is considered as one of the most successful semi-intensive shrimp farm in the world. With a production of more than 3.000 tons in 2009 (over a total of 780 ha of ponds), AQUALMA concept is entirely based on "sustainability" and "Corporate Social Responsibility" actions.

Dr Marc Le Groumellec is the manager of Aqualma Hatchery and one of the founders of the Aqualma firm. Veterinary by formation, Dr Le Groumellec is, first of all a scientist by sprit. He is in charge on the Sustainable Development Research Issues. In a previous research on Fishmeal replacement, Aqualma has tried out with soybean-meal (SBM). The result was interesting in term of growth, but it is difficult to avoid the risk from GMO (Genetically Modified Organism) affected on SBM (especially among SBM from Brazil) which is an ethic constrain for Aqualma policy. On the other hand, using SBM would compete directly on human sources of food (ethic food-base transfer) or other animal feed base that competition will causes on the cost production. While maggot as BSF larvae biomasses (Black Soldier Fly larvae, insect Hermetia illucens) are made from agro-industry by-products or from many others types of organic wastes, the concept would provide a best eco-image from the use of Maggot-meal.

Mutual goals of interests for BRKP- IRD- AQUALMA based on the <u>WIN-WIN principle</u>

The joint-research agreement should be discussed and established by the three party's agreement between BRKP/IRD/AQUALMA. It must be based on **the mutual goals of interest (the WIN-WIN principle)**, on a symbiosis force putting in common, knowledge and competences in order to reach the expected goals.

- Goal interest for Aqualma Aqualma is a rare company who attached strongly to the concept base of sustainable development summarized by three word started with letter P: "Planet People Profit". Using Maggot-Meal to replace Fish meal concerns the three goals in the same time. Fish meal from Peru could not be indefinitely available, increase of demands vs limited resources will drive the over fishing from natural stock. That is Planet Concern. The increase of fishmeal price has put many shrimp business in a bad economical situation. The tryout with maggot meal may reduce the production cost and may maintain a stabilized price for consumers. That concerns the goal of People and Profit. Another motivation of Aqualma is to be the first challenger on using maggot-meal on shrimp feed formulation. The tryout may extend to replacing Artemia by minilarvae to feed during the post-larvae stage.
- Goal interest for Indonesia The involvement maggot program in shrimp feeding is a good opportunity to valorize to maggot and giving more value added on maggot and PKM (Palm Kernel Meal). The research program may have a huge interest for Indonesia where shrimp culture is one of the most important aquaculture products for exportation.
- For IRD, the wish of Aqualma to use maggot in shrimp farming is unexpected recognition of interest on bioconversion research program especially from a professional big scale Industry.
- For IRD/BRKP research team in BRBIHAT, the direct benefits from this collaboration with Aqualma would be: the acquisition of specific equipment flike vacuum Packaging (see documents attached). the program would provide 30 tons of PKM. Infrastructure and equipment to transformation Unit from maggot fresh to maggot meal. Lab equipments, computer (note book) destined for Indonesian partner including a trip (evaluation mission after 6 months of tryout) to Madagascar with IRD scientist. All costs (travel+ accommodations) will be under the project budget evaluated at 30.000€ (thirty thousand Euros) see detail in annex.

In practical terms, the project must started by an **expertise trip** to Madagascar with purpose to analyze the feasibility of Bioconversion in a new context conditions. That was the reason of last mission from 16th to 30th of October. From all information's elements collected (presence of insect Hermetia, availability of PKM) the mission must be able to propose the step 2, the research designs and approach to be conducted to reach the definite goal. The competence and role of each party must be precisely described including the limit of respective responsibilities. The duration of the research tryout would be determined. Another trip (so called an **evaluation trip**) is planned with an Indonesian scientist partner, to evaluate together (BRKP/IRD/AQUALMA) the tryout results and to make a common conclusion as well as to determine, if any, the future development on maggot-meal in shrimp culture.

To gain time, and especially because of the raining season coming soon on December, Dr Marc Le Groumellec, has suggested me to make the **expertise trip** in Madagascar early in October. That is why the reason of my visit to Madagascar from the 16th to 30th of October 2010. This document will report my trip

which is separated into two schedules: (1) on the East Coast (Ocean Indian side) precisely at Anthalaha North-East coast, where there are two Projects, one on Palm Oil industry Plantations and another on Coconut plantation industry and (2) on the West Coast near Mahajunga to visit Aqualma Complex Shrimp Farm.

The first trip, considered as an expertise trip to make an evaluation of the feasibility of maggot Bioconversion in Madagascar. The purpose is to confirm the presence of Black Soldier fly (Hermetia illucens) that have already mentioned in bibliography and to evaluate the availability of Palm Kernel Meal (PKM). In this focus others sources of organic wastes usable as culture media base for bioconversion process would need to be identified. This is the first stage of expertise-evaluation mission, before taking any actions (establishing the protocol agreement on this joint-research program). The mission would be also for identifying the eventual difficulties and problems faced in the Madagascar context.

Visit at Anthalaha (North-East Coast)

Investigation on Palm Kernel Meal (PKM)

It is an excellent agriculture region explained by it high rainfall (2200mm) and a fertile soil. (60 km South of Anthalaha) is an area where in the recent past, a palm oil industry plantation (6000ha) have been tryout since 1989. But, from unknown reasons (bad conditions of the access roads, land locked areas, bad management etc...) the whole plantation are today unfortunately abandoned. After 5 hours trip from Anthalaha, Dr Le Groumellec and I, we have found a disappointed scene: the infrastructures and equipments (grading machine, collector's tractors, oil extractor unit, etc) are all irreversibly broken down. The palm trees, without maintenance and fertilizer input, are no longer productive and invaded by the natural vegetation. Very few, remained poorly productive, are exploited freely by the surround population groups. CPO (Crude Palm oil for cooking) is manually extracted. However, the PKO (Palm Kernel Oil) which resides in seed of palm fruit is not exploited and are thrown away. This is the part of the palm fruit that we are looking for, to exude the kernel and to extract the PKO. The PKM (Palm Kernel Meal), the solid rest of Kernel remained after PKO extraction, will be used as culture media for Maggot process.

In order to estimate the quantity available of Kernel produced, we have proposed a contract with the population to buy the kernel that normally thrown away with the seed. The work consists of crashing manually the hard shell nut and pickup the inside kernel. The price agreement (price by bag of 50kg kernel + transportation cost included from Ambohitralanana up to Anthalaha) has been calculated accordingly to the local workmanship payment with a significant additional tip service to motivate the work and get faster the idea of the availability of kernel in the region.

Investigation on Coconut meal (CNM)



Coconut meal (CNM) is a by-product from copra after extraction of Coconut oil (CNO). Like PKO and CNO are the only two lauric fats (C:12) in world trade. Its major fatty acids are on average C12:0 48% (lauric Fats), C14:0 18% and C16:0 9% by mass. Therefore PKM and CNM are very similar to each other by their properties and their composition. In case of lack of PKM, CNM may play as a substitute to produce maggot by bioconversion. Up today, no one has warred on this organic waste. The second visit in the region of Anthalaha is **Sambava** coconut

plantation. It is an agro-Industry of Copra situated on the north of Anthalaha. The first the impression after contact, we can say that the business is not working well. The whole plantations are lack of maintenance, a

similar image recorded on the palm oil industry at Ambohitralanana, but however, the infrastructures are less damaged. The causes are multiple but one is sure, it was came from the big storm on the March 2010 has destroyed a huge part of the domain. We have visited also the coconut fruit expel (by manual) and the . It is not working at the time we passed by. But the whole engine seems still in a good condition. The production recorded in 2006 was 6000 tons of copra, 2000 tons of oil extracted, and 3000 t of CNM. By a possible arrangement, the unit may be used for PKO extractor from Palm Kernel collected from Ambohitralanana and produce therefore the PKM.

Other by-products harvested seasonally from local fruits are possible like Lychee seed, Jatopha seed, Anacardium fruit, may take part in Bioconversion in Madagascar. The by-product from capture fish could be also considered as important. But at the end conclusion, the potentiality of all organic wash remains, no matter how, very limited..

To make a rapid conclusion on the availability of raw material for bioconversion, to produce maggot, it's clear, compared to Indonesia (the quantity of PKM and Copra Meal alone) Madagascar remains poor, very far below. The only possible solution is to process by importation.

To confirm the presence of *Hermetia illucens*, we have process by investigation with the population by showing the key ring containing BSF brought from Depok. Almost all people have recognized the insect but have made confusion with warp. To confirm the presence of BSF, we have processed by putting traps with palm Kernel seed crashed, but the time was short to allow the fermentation processing to produce the aroma to attack the Black Soldier Fly (BSF) and to make evidence of it presence.



Visit Aqualma Domain of Shrimp Farm (West Coast)

The Aqualma Farm is located on the West Coast of Madagascar. It is a complex composed by four units:

- (1) The site of **Moramba** is the breeder stocks preservation place of *Penaeus monodon* Madagascar's strain. The role of this site is to produce the first stage of shrimp larvae (Zoë stage),
- (2) The site Mifoko, the post-larvae unit production,
- (3) the site Mahajamba bay is the growth-out farm (780ha) and
- (4) The Besakoa site is an important unit for conditioning the final products (marketable size shrimp harvested from the growth-out farm) ready for expediting for marketing. An impressive example of shrimp culture industry, well experimented, keep going on the perfection following a strong guideline based on the concept of sustainability with respect on environment and on the principle of the CSR (Corporate Social Responsibility).

The breeders stock-management – hatchery and post-larvae production

The breeder's stock management represents the heart of the Industry business. The breeder's stock are carefully preserved with an extreme awareness conditions. In the same base location, the production facilities of the first stage Zoë of *Penaeus monodon* are produced. The selections (genetic and mass selections) are permanently processed with a solid genetic traceability. When the larvae have reached the stage of meta-zoë, the production of larvae will transfer to the second



hatchery (**Mifoko**) where the larvae will growth up to the post larvae 9 and then transferring to growth-out farm (Mahajamba).

At the level of this hatchery, the use of imported Artemia represents the main costly input. From Dr Marc Le Groumellec point of view, the micro larvae of BSF and the Mini-larvae could play an important role to replace Artemia in shrimp farming. This is an opportunity to promote the minilarvae and to provide a better value added to our product and the release from Artemia dependency.

The growth-out farm (Mahajamba)



The location is stated on land so called "Tanne", unfertile land zones, on the brackish-water onshore. No pond creations are built on the mangrove zones. A "Mega" pumping station (see photo in attached annex), are well done and intelligently designed. The unit pond size average is averaged from 6 to 8 ha each. The whole domain exploited areas is estimated at 780 ha visible from satellite Google Earth image. This is the point that makes Aqualma way of shrimp rearing, different from the rest. The

culture modality is a semi-intensive method where the density of shrimp

is restricted from 5 to 10 per m². Dr Le Groumellec has said "the low density make shrimp happy therefore good health, growing fast and produce a better test and flavor".

The harvest is occurred almost every day. During my passing-by the 27th of October, I have assisted the harvesting on the pond n° 69. An opportunity for me for the first time to see how Aqualma take care and respect the animal well-fare like the immediate **Slaughter by salt water ice -10°c** (term used to describe the killing and butchering of animals



usually for food) and hygienic routine practice considering the consumers safety.

Besakoa Unit Base of the post-harvest processing (packaging conditioning system)



From the growth-out farm, the biomasses of harvested shrimp are transferred immediately for conditioning and packaging process where the hygienic conditions are extreme. Systematically all packaging boxes are labeled for the traceability of the products started from the breeders up to the growth-out pond. The Packaging boxes are printed including the brand design and presented accordingly to the diversity of the consuming way and the marketing demands (Europe or Asia).

A real Corporate Social Responsibility (CSR) actions

The visit was ended by visiting the CSR aspects that Aqualma involved through the population of the village **Besakoa** where almost the whole Aqualma personnel are living there. The first actions are focused on populations Healthcare (construction of a complete dispensary, well equipped from dental care, maternity

up to ordinary care). Three practicing doctors are permanently resident at the locality. In case of any serious emergency, the evacuation may be done by plane from Aqualma small air port near-by the Besakoa.

Another action of CSR program, is concerned the education. A primary school is well maintained and equipped by a rich library donated by French Embassy in Madagascar. A new High-school is recently built by the Aqualma partners in France (Intermarché).

All by-products (shrimp shell and head) are well managed into compost and used as biofertilizer for the village kitchen Garden. During the visit of the compost areas, BSF Hermetia has been. And at the same time the yellow flower, *Sphagneticola* sp has also been met at the high-school garden. That has giving us more confident on the feasibility of bioconversion nearby the locality of Besakoa village.



Conclusion of the mission:

According to what we have seen, it is clear that the bioconversion may be processed in Madagascar. The climate conditions are similar compared to Indonesia -Sumatra. But the negative point stills the lake of PKM to allow to the maggot to be produced in Madagascar. However the strategy and approach will be slightly modified compared to what we have done in Indonesia.

Dr Marc Le Groumellec, 42 years old, is a veterinary formed in France (Nantes) a strong back ground for animals care. His engagement with shrimp has started since 1992 after university graduation by involving the shrimp culture program at Tahiti under IFREMER French research Organization. After four years of experience, Aqualma has proposed a post in the Firm that has conducted him to become the founder of Hatchery of Aqualma. The first priority was focused on the hygienic conditions and disease issues and the healthiness of the animal. But overall, the quality of Dr Le Groumellec is his state of mind as scientist futurist planner, anticipating the coming problems. The lack of fishmeal issue and his intention to use maggot to replace fishmeal, have illustrated clearly his awareness on the future problems. The success of his carrier based on his 12 years of experiences in the shrimp culture is precious to be shared profitably for all of us throughout this collaboration and benefit for Indonesian shrimp experience.





Pumping unit composed by 12 huge "caterpillar" pumps. Each pump has a debit capacity of 2 m^3 per second. For the whole 12, the water flow debit is around 24 m^3 per second.

Dr Marc le Groumellec (first from the left). Background of the photo: View of a 8 ha pond unit.



Shrimp harvested from a semi-intensive culture mode with a density of 5 invidious per square meter. The average size of pond unit is about 7 ha. The whole domain contains 780 ha. Yield production shrimp estimated for 15 tons per pond unit.





Proposal of the budget distribution

	Items	Value in €
1	VACUUM BAG SEALER AZ-450E-220V (NOZZLE TYPE) + consommables	5 500
2	Weight 250 kg	
3	Equiments boiller + dryer (from maggot frash to maggot-Meal)	
4	Equipments Laboratory (balance, adapter binocular)	
5	Computer - Note Book	1 000
6	30 tons of PKM	3 000
7	Analysis Biochimistry(external services standard ISO IPB Bogor)	3 000
8	Expedition (maggot meal to Madagascar)	2 000
9	Evaluation trip to madagascar for two persons (IRD/BRKP)	5 000
10	Others	2 200
		30 000

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Top A B C D E F G H I J K L M N O P Q R S T U V W Y Z

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When food is vacuum packed in bags, the effect of the pump is highly visible, because the bags will shape themselves tightly around the food. Not so when vacuum packed in a jar, which is when a vacuum gauge is most helpful and will keep the jar from imploding.

In order to maintain the vacuum, containers are constructed of special materials which provide an oxygen barrier.

Тор

Vacuum packaging is not a substitute for canning or dehydration

Vacuum packaged food will taste fresher and last longer than food stored in conventional containers.

But because the food is not devoid of moisture or potentially lurking pathogens, it is important to remember that vacuum packaging is not an alternative to refrigeration.

Of course you don't need to refrigerate vacuum packaged cereal. But you do have to refrigerate meat, dairy and other products that require it.

Тор

Shelf life of vacuum packaged foods

Food	Stored In	Normal Shelf Life	Vacuum Shelf Life
Large cuts of meat: beef, poultry, lamb and pork	Freezer	6 months	2-3 years
Ground meat: beef, poultry, lamb and pork	Freezer	4 months	1 year
Fish	Freezer	6 months	2 years
Coffee beans	Room temperature	4 weeks	16 months
Coffee beans	Freezer	6-9 months	2-3 years
Berries: strawberries, raspberries, blackberries	Refrigerator	1-3 days	1 week
Berries: cranberries, huckleberries, blueberries	Refrigerator	3-6 days	2 weeks
Cheese	Refrigerator	1-2 weeks	4-8 months
Cookies, crackers	Room temperature (periodically opening)	1-2 weeks	3-6 weeks
Flour, sugar, rice	Room temperature	6 months	1-2 years
Lettuce	Refrigerator	3-6 days	2 weeks
Nuts	Room temperature	6 months	2 years
Oils with no preservatives, like safflower, canola, corn oil	Room temperature	5-6 months	1-1.5 years
Wine	Refrigerator	1-3 weeks	2-4 months

Table adapted by Tilia Inc. from Dr. G.K.York, Dept. of Food Science & Tech, U of California, Davis.

Top







Nozzle Type Vacuum & Gas Flushing Packing Machine

Introduction

Intrise Co., Ltd is a manufacturer of nozzle type vacuum packaging machine over the world.

Having a foreign corporation in Japan and many distributors worldwide, Intrise exports to more than 30 countries including Europe (Germany, UK, France, Finland, Sweden, Netherlands, Slovakia, Russia, Swiss, etc), America (USA, Canada, etc), and Asia (Japan, China, Singapore, Malaysia, Indonesia, etc).Oceania(New Zealand, Australia) and Middle East.

Intrise is a company with cutting edge technology, manufacturing products based on precise and aggressive drawing system using 3D graphics and with engineers with wide experience in related fields.

Intrise is a reliable company both quality wise and business wise. The company has been chosen as Small & Medium Business Export Prospect, Venture Company, Inno-Biz Company, and Corporation Research Center by the government and various institutions. 

Corporation Research Center continues R&D to improve the functions and quality of the product.



AZ-Series









Packaging process







Vacuum



Gas Flushing



AZ-450E

- Compact design for the desk use
- Weight : 30kg
- Suitable in small work spack

AZ-450ES

- Stand attached type
- Weight : 60kg
- Superior mobility using the caster attached to the stand

AZA-450ES

- Upper body's angle control type (0~45° by handle)
- Weight : 65kg
- Recommended for liquid/grain packaging

AZH-450ES

- Work tray's height control type (150mm from the sealing bar by handle)
- Weight : 75kg
- Recommended for packaging products with various height (150mm)

Options

- Hot-wire : Single or Up & Low
- **Sealing length :** 450, 600, 800mm
- Printer type : Prints manufactured date and expiry date
- Vacuum pump : Ejecotor, Oil pump , Oilless pump (selectable)
- Controller : Normal type/Mode type (Max. 19 types)
- Filter type : General, powder, liquid type filter (selectable)
- Long nozzle : Adjusting nozzle length possible Max.(100mm from the sealing bar)
- 1 ph/3ph, 220/110V, 50/60Hz
- Air cleaner : Cleaning inside vacuum line by blowing out any particle with compressed air.

Sealing