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Edible insects as feed ingredient:
Nutritional and environmental aspects - Food safety and legal requirements

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1. Introduction

The world's population is continuously increasing and accordingly, demand for food is growing. The United Nations Food and Agriculture Organization (FAO) estimated that by 2030 over 9 billion people will need to be fed with billions of livestock (ANSE, 2015). And by 2050 the world will have to produce an additional 70% food compared to that of 2011, responding to the growing population (IFIF, 2012). The International Feed Industry Federation (IFIF) believes that the meat production will double by that time (Veldkamp et al., 2012). The FAO has therefore been emphasizing a potential problem of the world's food and feed security. The issues of food security are of course a huge concern for everybody on this planet, but it should be perceived even more acutely in developing countries (ANSE, 2015). In developed countries, where food security is not a main concern, insects are suggested as a possible solution of health problems related to food; food safety and environmental sustainability, which are resulted from prolonged shelf life of food and agricultural intensification (Belluco et al., 2013).

In January 2012, an expert conference meeting on the subject "*insects as food and feed*" was held in Rome by FAO of the UN, the Dutch government and Wageningen University (WU) of the Netherlands. The conference conceded a promising potential to insects as a sustainable raw material for production of food and feed, particularly rich in high quality proteins (Veldkamp et al., 2012). On the following year, FAO/WU published a paper (2013), recommending to rear edible insects on an industrial scale. Many industrialized countries, which are not accustomed to eat insects, have been discovering the great potential of insects as human food and animal feed since 2012.

The idea of edible insects as feed ingredients started from a simple fact. When Sushi was first introduced in Western countries, eating raw fish was not accepted by a large majority of the population. 20 years after, these pieces of raw fish are considered as a delicacy, have become a part of the western dietary culture as well. We can take the bet that in ten years' time, insects may be found on our table as an everyday base meal. Meanwhile, edible insects will surely and are already beginning to be substituted partially in the diet of livestock with the advantage of needing less energy to be produced, allow a better valorisation of raw materials and induce low environmental costs. Moreover, insects intrinsically have always been parts of the natural diet of aquaculture and poultry species.

The corresponding challenges remain in "*how to scale up insects into the quality and quantity needed for the current animal feed industry*". The traditional way of capturing of insects from

the wild is not safe anymore due to environmental pollutants and the risks of microbiological infections. Insect farming on an industrial scale is able to allow greater control of the hygienic practices of rearing and makes sure safe feed sources to be given to insects, which will consequently mitigate potential microbiological hazards.

There is then a large variety of tasks to be developed, among which the selection of suitable insects, developing economical feeding substrates, automation and optimization of the rearing procedure and an appropriate sanitary control. Another important challenge has to be won as Western legislations are very conservative towards a new source of food and feed. Therefore, a growing development of that new feed chain in Europe will only be possible with the establishment of new regulatory frameworks and a re-assessment of the food and feed safety as well as ethical practices considering animal welfare.

The aim of this study is to investigate the most urgent tasks: a food safety and legal requirements for the industrial feasibility of edible insects as food and feed ingredients as well as their nutritional and environmental values.

2. Insect industry

Approximately 1.8 million different species of animals are living on Earth and three fourth of them, about 1.3 million species are insects (Choi, 2011). Based on genome sequencing data, the class of insects originated on Earth between 350 and 400 million years ago (California Academy of Sciences, 2014). The oldest insect fossil known, *Rhyniognatha hirsti*, emerged very early during the Devonian period, around 380 million years ago when the first terrestrial ecosystems were formed on Earth (Engel & Grimaldi, 2004). According to Choi (2011), there are about 15,000 species of insects directly or indirectly being related to humans. These species have also been classified into beneficial insects or pests from the perspective of humans (Kim, 2000). Although humans have been utilizing insects as resources in everyday life for a long time, some insects are still regarded as pests that are harmful to humans and crops, and hence a large variety of insecticides or anti-parasitic chemical substances have been developed to eradicate them.

These recent years, insects have been revalued as untapped living resources on the planet with a great potential for bringing high value-added resources to industry. “*Industrial insects*” are defined as any type of insect that creates added value in industries (Yu et al., 2014). Entomologist John Losey of Cornell University high-lightened the importance of insect resource in economy as through their work of dung burial, pollination, pest control and wild nutrition, they contribute at the least 57 billion dollars annually to the U.S. According to the Rural Development Administration of South Korea, the world’s market scale of insect industry is expected to grow up to 38 billion euros in 2020, starting from 11 billion euros in 2007. Additionally, it is expected that the convergence and integration of technology will accelerate the practical application of insects in diverse industrial fields.

Insects, which constitute the three fourths of all animal species, have an important role as parts of the biosphere on this living planet (Kwon, 2000). Until now, a significant part of them has been considered as crop pests competing with humans for food. On top of that, many children or even adults still ask “what is the goodness of insects” This clearly indicates that a majority of people tends to associate insects and negative things such as disgusting appearances, biting, stings, diseases and crop destruction. Of course, there are undoubtedly harmful insects, such as mosquitoes which may transmit diseases like malaria. Nevertheless, many of them have been valued and regarded as beneficial all along throughout the human history (Defoliart, 1995).

2.1 Examples of insect industry in the past: Sericulture, Apiculture and Cochineal dye

a) Sericulture

The classic examples of industrial application of insects are sericulture and apiculture. Sericulture, which originated in China between 4,500 B.C. and 2,000 B.C. (Vainker, 2004), uses the caterpillar of the domesticated silk moth (*Bombyx mori*) to produce the silk used to spin its cocoon. Silkworms have triggered the cottage industries worldwide, and consequently have been the subject of intense domestication due not only to technological potential, but also to nutritive value (ANSE, 2015). Several countries including China and India have invested in the production of silkworms for obtaining silk and pupae, the latter used as food and feed (Defoliart, 1995). Even in South Korea, where there is no specific diet habit nor culture of edible insects, silkworm pupae are being sold as snack in the streets or found in the supermarket as can-conserved. Since 1987, the Thai Ministry of Public Health has approved the incorporation of silkworm pupae into the feed formulation for children suffering from malnutrition (Defoliart, 1995). In some countries like India, Japan, Sri Lanka and China, residues of pupae of *B.mori* are being utilized as feedstuffs directed to fish and poultry (Kiuchiand & Tamaki, 1990).

b) Apiculture

Another example of a successful application that has become an important industry is apiculture. Honeybees (*Apis mellifera*), whose domestication was succeeded by Egyptians between 3,500 B.C. and 2,500 B.C., are kept in artificial hives from which hive products are collected (Lokeshwari & Shantibala, 2010). Beekeeping was first practiced for honey production and then other useful products from bees, such as beeswax, pollen, propolis, royal jelly and bee venom are commonly gathered and sold (Schmidt & Buchmann, 1992). Honeybees are also the most important vector to operate pollination between flowers and this is perhaps the best known ecosystem service performed by insects (McGregor, 1976).

c) Cochineal dye

Cochineal, an insect from the Dactylopiidae family, is not very well known by the public but still is the most widely used insect in agro-food and cosmetic industry (ANSE, 2015). Carmine, also called E120, is the natural bright red-colored pigment extracted from carminic acid (Dapson et al, 2007). Cochineals produce carminic acid as a deterrent against their predatory insects (Belluco et al., 2013). Carmine is one of the few natural colorants that resists degradation

with time, furthermore it is the most light- and heat-stable, and even is a more stable colorant than some synthetic food colors (Acero et al., 1998). The cochineal dye was first used by the Aztec and Maya people of Central and North America for coloring fabrics (Taylor & Dormedy, 1998). The demand for carmine has sharply decreased due to the invention of synthetic colorants in the 19th century (DiCello et al., 1999). Recently, consumers seem to prefer the natural E120 colorant to synthetic colors, yet most of these consumers are not aware that carmine is derived from insect cochineal. Although carmine is not suitable for vegetarians nor allowed in some religions, it is authorized as a dye by European regulations and used in various food formulations such as yogurt, candy or sodas (Cardon, 2003; Verkerk & Tramper et al. 2007).

2.2 World's current status of insect industry

These creatures, small in size and short in life are not only present throughout the world, but also have been utilized in daily life of humankind. Despite the world's high degree of technology, people just started to recognize their value on both industrial and economical aspects (Choi, 2011). Moreover, after the UNCED conference in Rio de Janeiro, 1992, an ecological interest for the preservation of biological diversity has been escalated in relation to insects, due to their role in the ecosystem as more than half of all described species on Earth are insects (Kwon, 2009). Therefore, an intensified competition has taken place between countries on researches and studies for the use of insects in various industrial fields (Lokeshwari & Shantibala, 2010).

Some developed countries have already started to establish a legal basis in order to foster the insect market as a future industry on their territory (Oh, 2008). For instance, Japan realigned its legal status relative to animals, "*Prevention of Cruelty to Animals Act*" for the pet insects and "*Staple Food Control Law*" for the hygienic control of edible and therapeutic insects (Choi, 2011; Jang, 2015). On the strength of these legislative supports, the Japanese government invested 20 billion euros in 2 years from 2002 (Choi, 2011). United States and the European Union also encouraged investments in the insect industry through regulations relative to microbial pesticides and legal management of insects as parts of flora and fauna (Yu et al., 2014).

The Netherlands, a front-runner in the world's insect industry, has achieved a significant increase in its agricultural exports (by 5.3 billion dollars in one year, between 2002 and 2003). This has been the result of 10 years of steady efforts started in the 1990s on technological

progress in biological farming and pest control using natural enemy insects (Choi, 2011). Moreover, in 2013, the Wageningen research unit of the Netherlands and FAO published a paper which encourages the contribution of edible insects for solving the world's food and feed security (IPIFF, 2014). In spite of a conservative European legislation relative to new foods intended for animal and human consumption, the Netherlands' government is investing a significant amount of money as a financial support on a project for getting insects into mainstream diets (van Huis et al., 2013). In 2012, The Netherlands have already invested one million euros on research and legislative preparation for governing industrial scale insect farms (Jang, 2015).

2.3 Current status of insect industry in the Republic of Korea

As a South Korean, it seems quite logical for me to investigate the current situations and trends of insect industry in South Korea. The exploitation of insect resources has been, up to now, confined to a few domains, such as sericulture, apiculture, and edible and therapeutic insects (Kim, 2000). Recently, the Korean Rural Development Administration has started to conduct studies and researches on pet and educational purposed insects, biological farming with pollen-mediated insects and natural enemy insects, biodegradation, and edible and therapeutic insects (Choi, 2011). Consequently, insects are being used as novel materials in a variety of industries since 2000 (Oh, 2008). The size of the Korean insect market was estimated at about 150 million euros in 2009, and is now expected to double in 2015 (Choi, 2011). Pollen-mediated insects still form the largest part in this field, as their share corresponds to approximately 34% of the total market and about 54 billion euros worth in 2009 (Choi, 2011; KEI, 2014). As for edible and therapeutic insects, the internal market has just been formed, but the prosperous future of this particular area is anticipated to grow up to 70 million euros in 2015 (Sah & Jung, 2012).

According to the Ministry of Agro-Food of South Korea, statistics in that country relative to insect farms are as follows, in 2012: a total of 395, out of which 232 breeding farms and vendors, 72 distributors, and more than 20 places for insect specimen and goods producers. There are also 59 insect gardens and experience centers altogether and 12 research institutions specialized in insects. In 2013, the number of breeding farms increased to 265, that is 30 more farms in one year, yet these farms are still being operated as small scale businesses.

In South Korea, no independent or state agencies nor research institutes were specialized in insect resources. Recently, local insect research centers have been established in every province to keep pace with the industrialization of insect resources. As for private institutions, most of them have been founded and are run by pesticide or pharmaceutical companies, but they only exist as laboratories in order to testify the efficacy and efficiency of insecticidal products. Facilities in colleges or universities are relatively well supplied for studying or research, but are still not enough equipped for breeding or raising insects. According to the Korean Research Institute of Bioscience and Biotechnology, in 2009, only 30 % of the total agricultural research institutions managed to equip themselves in a breeding or rearing system for insects. Lepidoptera larvae are raised and utilized mostly for testing the insecticidal efficacy and the study of natural enemy insects. Diptera and Coleoptera are utilized for research on parasitic, predatory and pollen-mediated insects.

One should remark that traditionally insects were not recognized as usual food or feed ingredients in Korea, so there is no viable technology nor specialized systems to produce a large quantity of good quality insects on a continuous basis. AgriProtein in South Africa and Enviroflight in the United States are examples of insect farms that have developed into an industry scale-farming (Veldkamp et al., 2012). On the same subject, the Netherlands have recently developed a specific supply chain including large scale-farming and marketing that are supported by research institutes, NGOs and the Dutch government (van Huis et al., 2013).

However, the technological level of the insect industry is self-assessed at a bit less than 80% compared to Japan (Kim, 2011). A patent share is defined as the percentage of a universe of patents owned or created by one subset of that universe, knowing that in this specific context, the universe is defined as the insect industry (Oh, 2008). According to the Patent and Trademark Office in 2010, Japan shared the most patents with 379 cases (33%), followed by United States (359 cases, 32%), South Korea (314 cases, 28%), and the EU (85 cases, 7 %) (Choi, 2011). Investors can get an idea of the status of an industry by examining industries that have their market shares growing in patent discoveries (Oh, 2008).

Despite high patent shares and originalities in new insect resources, the national Korean insect industry is still too tenuous to create positive effects on agricultural income and economy. Considering that diverse markets through the local and online are not included in the databases, the volume of this industry may be larger than documented. Nevertheless, it has been strongly criticized that Korea had no independent state agencies nor institutes to conduct large scale

researches on insects and, moreover, no legal basis had been put in place. But, in Feb 2010, the Korean government finally promulgated the first legislative bill encompassing regulatory supports to rearing farms, grants to their legal status and also investment on human resources. Now however, challenges remain on the industrial convergence with technological advancement as well as the expansion towards overseas markets. Efforts should be extended to intensified researches on various insect-based resources and exploration relative to useful insects.

2.4 Trends of insect industry

Insect industry around the world shows very distinct trends depending on the current situation that each country is being faced with. In addition, the overall tendency differs according to the different cultural backgrounds. In East Asia, including Korea, the tendency is to focus on edible and medicinal insects particularly directed towards humans, whereas the US and Europe concentrate on pollen-mediated insects and natural enemy insects. Recently, more and more people worldwide are conscious of the importance of pro-environmental life-style. Restrictions for the use of chemical pesticides including insecticides have become much stricter than ever in most developed countries. Besides pollen-mediated and natural enemy insects, considerable research has been conducted to understand and exploit some insects (fly larvae and beetles for instance) for bio-degradation, since these types of insects feed on organic side streams and digest them, with a transformation into bio-fertilizer and protein sources as they grow into larvae and pupae. As an alternative and sustainable protein source, edible insects are no longer considered as hideous and an outdated culture from some Eastern parts of the world, but are likely a crucial key to solve at the same time the world's food security and safety.

Domains of the insect industry have become diversified along with the development of that industry. These different domains are discussed below in connection with current trends. A classification could be the following:

- agricultural use (insects as natural enemy, pollination insects);
- recreational, educational and tourism purpose;
- bio-degradation using insects;
- medicinal and therapeutic purposes;
- bio-mimetics and bio-technology;

- edible insects for food and feed.

a) Agricultural use as insect natural enemies and pollen-mediated insects

In Europe, there has been a continuous growth for the last 20 years of biological farming, typically using predator insects and pollen-mediated insects for pest control (Choi, 2011). Biological control begins at first with searching natural enemies of the pests to be controlled, for instance insects such as predatory beetles, bugs, gallmidges and mites (Kim, 2000). These insects are used to control pests and diseases in crops instead of applying chemical insecticides or pesticides (Oh, 2008). Among EU companies developing such insects, the Netherlands' Kopper Biological System is globally well known and its annual turnover is somewhat around 20 million euros (Choi, 2011). In 2003, the Netherlands reduced the use of chemical insecticides by 65% thanks to biological control with insects (Kwon, 2009). Recently, extensive researches and studies have been made relative to micro-organisms, bio-stimulants and pheromones as they could be more efficient solutions to improve plant resistance and resilience (Jang, 2015).

Insects are the major pollinators of plants, including of course food plants but their number has dramatically declined due to the environmental pollution, among which the extensive use of chemical insecticides (Choi, 2011). Such a negative evolution has increased the demand for pollinating insects (Kwon, 2009). Artificial pollination is a labor intensive task but absolutely needed for a good fruit production and quality. From the late 1980s, Bumble bees have been used to fertilize many food plants, especially tomato crops (Oh, 2008). South Korea used to rely on 100% import for Bumble bees as natural pollinators. Since 2005, the country succeeded to mass-produce *Bombus terrestris* that actually replaces almost 70% of Bumble bees for domestic needs (Choi, 2011). Recently, flies are being used for the pollination of seed-producing crops.

b) Recreational, education and tourism purposed insects

Japan is well known for its huge market of pet insects that has been developed from the early 1980s. The market scale for the pet purposed stag beetles only reached about 2 billion euros in 2009 (Choi, 2011). Throughout Japan, there are more than 1,000 specialized insect-shops providing customers with various species of insects (Jang, 2015). The boom of pet insects is expected to continue with the expansion of retail trade and shops such as pet food retailer, pet item retailer, animal retailer, and pet related services.

The best example of tourism purposed insects may be found in the UK and the Channel Islands as some 30 different Butterfly houses and gardens are being operated as an eco-tourism associated with environmental education (Choi, 2011). These places succeeded to restore the ecosystem for insects, typically in outdoor gardens, and also serve as eco-experimental centers.

c) Bio-degradation

Some insect species, like the black soldier fly (*Hermetica illucens*), the common housefly (*Musca domestica*) and the yellow mealworm (*Tenebrio molitor*) can be reared on organic side streams, such as manure, pig slurry and compost (van Huis et al., 2013). They naturally feed on such organic wastes and bio-transform them into bio-fertilizer. At the same time, insect larvae produce protein sources as they grow into either larvae or pupae stages. Therefore, such insects are expected to complete the economic and environmental cycle of the food and feed chain (Veldkamp et al., 2012).

“*Ecodiptera project*” was launched to recycle animal wastes, especially pig manure across the EU in 2004 (van Huis et al., 2013; Veldkamp et al., 2012). Larval flies are reared on solid manure to produce bio-fertilizer and protein rich food. In Slovakia, a pilot plant for bio-degradation of pig slurry has been implemented with development of methods for the maintenance of fly colonies under optimal conditions (Veldkamp et al., 2012). Agriprotein Technologies of South Africa also established a pilot plant where insect-based proteins (Magmael) and oil (Magoil) are produced from nutrient recycling (van Huis et al., 2013).

Nevertheless, rearing insects on organic side streams is not legally permitted by the EU food and feed legislation as up to now there are potential risks in the transmission of pathogens and contaminants from organic wastes via insects (van Huis et al., 2013).

d) Medicinal and therapeutic insects

Insects have been used since centuries to cure human diseases and the word entomotherapy may be used. Li Shizhen’s Compendium of Materia Medica, which is one of the largest and most comprehensive books on Chinese medicine (1368-1644) until now, listed approximately 300 medicinal insects species (distributed in 70 genera, 63 families and 14 orders) that have been used as entomotherapy (Choi, 2011).

Fly maggots have a great potential in a variety of fields thanks to their biological properties. Besides their nutritive value and a capability of bio-degradation of organic wastes, they also have been used therapeutically to clean out necrotic wounds, and this method for healing has been approved in 2004 by FDA as a medical advice (van Huis et al., 2013).

Another example of entomotherapy refers to the positive effect of chitin in poultry feed. The ESBL (extensive spectrum beta lactamase) bacteria have an enzymatic property to break down beta lactam anti-biotics. The prevalence of these bacteria in the livestock is quite high due to the over-use of such anti-biotics. A study revealed that about 94% of the chickens reared in the Dutch poultry farms and subsequently found in the Dutch supermarkets are infected with bacteria having ESBL genes (Belluco et al., 2013). As chitin and chitosan of insects' exoskeleton has a variety of biological properties including reinforcing effects on immune-defensive system in body, insects chitin is thus added to their food as an immune-stimulatory adjuvant and make "*the use of antibiotics superfluous*" (van Huis et al., 2013).

3. Edible insects as feed ingredient

3.1 Edible insects as feed: Backgrounds and current status

The International Feed Industry Federation (IFIF) estimated the scale of the global compound feed production equivalent to 870 million tonnes in 2011 (van Huis et al., 2012). The Food and Agricultural Organisation (FAO) believes that by 2050 the world will have to produce an additional 70% food compared to that of 2011 (IFIF, 2012). As the world's population is growing in wealth, it usually accords with an increased consumption of higher quality animal proteins (Veldkamp et al., 2012), and correspondingly the meat production is expected to double (Vrij, 2013). The scarcity of raw materials for protein production will be accelerated as the world's population is continuously increasing. Nevertheless, improvements in food producing system, such as intensive farming policies, genetic selection and genetically modified organisms (GMOs) have increased the food yield, but also led to negative impacts on environmental sustainability and animal welfare (Belluco et al., 2013).

Currently, the main protein ingredients in animal feed are fish meal, soybean meal and processed animal meal, but the use of processed animal protein in livestock is currently forbidden in the EU due to the TSE legislation following the BSE crises in 1996 (Veldkamp et al., 2012). Raw resources for soya cultivation, including land and water, are limited worldwide and small pelagic forage fish, from which fish meal and fish oil is derived, have been markedly reduced due to marine over-exploitation (Vrij, 2013). The growing scarcity of resources correspondingly increased demand for raw materials and their price has doubled for the last ten years (Veldkamp et al., 2012). In the Netherlands, the price of fishmeal increased from \$ 650 per ton to \$ 1,410 between 2002 and 2012 (see Figure 1). So, an approach to search for new, sustainable alternatives was inevitable. The idea of insect meal has risen among other possible raw materials, such as duckweed, algae and lupines by the fact that insects have a high protein level as well as a low cost of production and ecological sustainability.

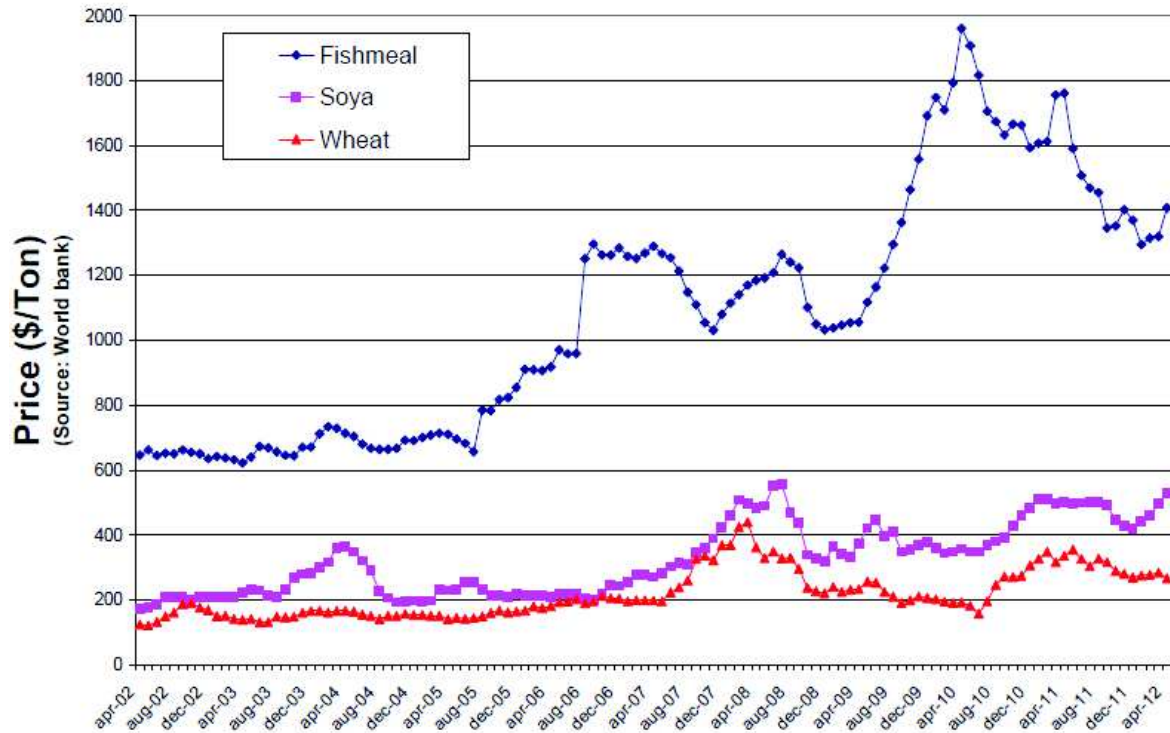


Figure 1. Raw material prices for fishmeal, soy and wheat of the last 10 years (Vrij, 2013).

In January 2012, an expert conference meeting on the subject “*insects as food and feed*” was held in Rome by FAO of the UN, the Dutch government and the Wageningen University of the Netherlands. That conference pointed out the great potential of insects as a raw as food and feed, due in particular to their high level in proteins (van Huis et al., 2013). In the same year, the Dutch Ministry of EL&I (Economic Affairs, Agriculture and Innovation) initiated a study on the feasibility of insects as sustainable food and feed source (Veldkamp et al., 2012). In the following year, the collaborative paper between FAO and the Wageningen University of the Netherlands, “Edible insects: future prospects for food and feed security of edible insects” has inspired related studies and experimental trials using various species of edible insects, but more importantly suggested many viable answers towards “how to scale up of insects into the actual quality and quantity for the current animal feed industry” (van Huis et al., 2013). The challenges remain in selection of suitable insect, economical substrates, assuring food and feed safety, and also automation and optimization of the rearing procedures under sanitary control (Belluco et al., 2013). This new feed chain should in all cases satisfy the establishment of new regulatory frameworks through a re-assessment of health risks well as ethical practices considering animal welfare.

3.2 Edible insects: nutritional values

There are 2,086 different species of edible insects have been consumed worldwide in connection with a variety of cultural and traditional backgrounds (Ramos-Elorduy 2009; Rumpold and Schlüter 2013). The FAO paper (2013) on edible insects as food and feed has elicited a number of researches and studies evaluating the nutritional value of insects. However, published documentations related to the nutritional evaluation have investigated a very limited number of species despite the 2,086 different species quoted above (ANSE, 2015). Therefore, the nutritional figures referred in publications should be taken with much care, knowing that there are always significant variations between different species.

Xiaoming and others (2008) gave an overview of the nutritional compositions of 6 different species of well-known edible insects (see Table 1). The data were calculated to the same amount of moisture at 8.2 %, which applies to 6 species of insects, 3 types of fishmeal and soybean meal. Insects are generally described with a high level in proteins varying between 37.5 % and 69.8 % while fishmeal and soybean meal contain 49.3 % to 66.1% of protein (see Table 1). According to Ayieko and Oriaro (2008), edible insects correspond to about 5 to 10 % of the total proteins consumed in some African communities. The high level of proteins corresponding to insects have made them being considered as an important source of proteins in certain human populations for a long time.

Source	Order	Latin name	Moisture g/100g	Protein g/100g	Fat g/100g	Ash g/100g	Reference
Mealworm meal	Coleoptera	Tenebrio Molitor	8.2	59.2	27.9	4.5	1
Termite meal	Isoptera	Macrotermes subhyanlinus	8.2	44.1	28.7	3.4	2
Cricket meal	Orthoptera	Gryllus testaceus	8.2	53.5	9.5	2.7	3
Grasshopper meal	Orthoptera	Caelifera sp.	8.2	69.8	7.3	4.6	4
Black soldier fly meal	Diptera	Hermetia illucens	8.2	38.6	32.1	12.9	4
Silkworm pupae meal	Lepidoptera	Bombyx mori	8.2	44.1	24.8	4.6	4
Housfly larvae meal	Diptera	Musca domestica	8.2	37.5	19.8	23.1	5
Krillmeal			8.2	59.2	18.4	13.3	6
Fishmeal (Herring)			8.2	66.1	9.2	9.5	4
Fishmeal (Menhaden)			8.2	56.9	9.2	18.4	4
Fishmeal (White fish)			8.2	56.0	3.7	22.0	4
Soymeal			8.2	49.3	1.9	6.8	7

Table 1. Overview of the composition of different kind of insects compared to some fishmeals and soymeal (Vrij, 2013)

As insects are considered as a significant protein source to replace fishmeal and soybean meal, it is crucial to compare not only the total content of proteins, but more importantly, their amino acid composition (see Figure 2). In other words, fish meal, soybean meal and insects may show very distinct amino acid compositions despite a similar total content of proteins. Figure 2 below demonstrates the composition of total essential amino acids corresponding to the previous quoted 6 different species of edible insects, 3 types of fishmeal and soybean meal. It clearly shows that the essential amino acids measured in the 6 species of insects are generally well-balanced to meet human needs (Raubenheimer and Rothman 2013; Rumpold and Schluter 2013a.).

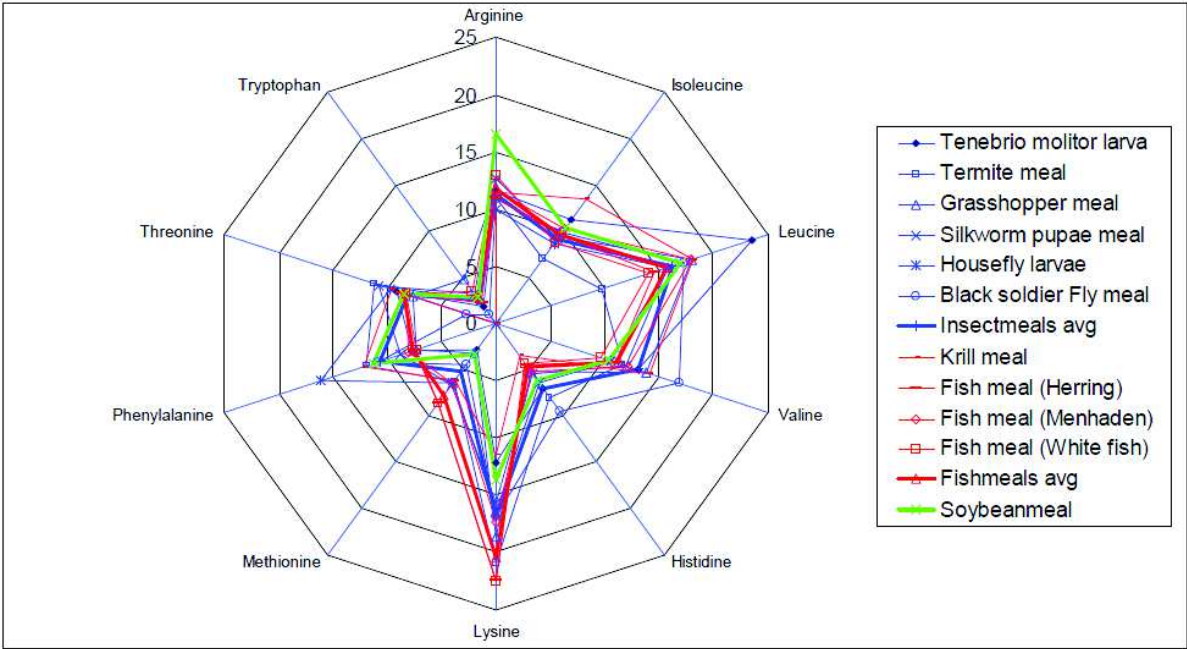


Figure 2. Amino acid composition (% total essential amino acids (100%)) of insect meal, fishmeal and soybean meal (Vrij, 2013).

Nutritional requirements for animal diet usually vary on a great scale according to species or races and even different developmental stages within the same species. Concerning insect meal for livestock, it is also necessary to consider other environmental factors during the rearing procedure, such as climate, habitat, soil, and substrates in their nutrition. Hence, the correct choice and use of specific insect species for the livestock is entirely dependent of farmers, and developers of the insect meal industry. Insects demonstrated in this study contain a higher level of methionine, lysine and valine compared to soybean meal (Vrij, 2013). Therefore, nutritional

demands specific to the livestock concerned will determine the choice of insect species or their combination.

The fat contents of the insect group ranges between 7.3 % and 32.1 % (see Table 1). Despite the fact that certain species of insects show a particularly high values in calories or lipids or minerals or vitamins, they commonly have a low content in carbohydrates. Besides their high content in proteins, insects show a very interesting trend in their lipids contents. Larvae and pupae show higher lipid contents compared to their adults (Chen et al., 2009). For instance, the orders of Isoptera and Lepidoptera tend to contain the highest level of lipids. Also, the fat contents in insects varies depending on the substrates they feed in their diets. Chen and others (2009) demonstrated that the lipid contents of insects, varying between 7 and 77g/100g of DM (dry matter), was largely dependent on their diet. St-Hilaire (2007) compared the lipid contents in the same species, Black soldier fly (*Hermetia illucens*) that were fed with different substrates in their diet and the ones reared on manure and fish offals had higher omega-3-fatty acids composition in lipid compared to the ones reared only on manure. This information is interesting as fish contains profitable ingredient for food and feed thanks to a high level of healthy fats, omega 3 and omega 6, together with a high digestibility (Vrij, 2013).

If the lipid composition can be manipulated in insects during the rearing procedure, it then gives another reason to promote insect meal over fishmeal. In addition, the healthy fats, PUFA (polyunsaturated fatty acids) are composed of omega 3 (n-3 fatty acid, rich in linolenic acid, eicosapentaenoic acid (EPA) or decosahexaenoic acid (DHA)) and omega 6 (n-6 fatty acid, rich in linoleic acid), and the recommended ratio between omega 3 and omega 6 is 1 to 3 (Belluco, 2013). Pereira and others (2003) proved that toasted pupae of silkworm (*Bombyx mori*) contains 32 % of PUFA, consisting of 7.03 % of linoleic acids and 24.4 % of linolenic acids, whose ratio is very close to the recommended ratio. In general, cholesterol level in edible insects varies from low to a level comparable with other animal foodstuffs (Ritter, 1990). Likewise, the cholesterol composition in insects will differ according to the different substrates present in their diet as insects are not able to fabricate their own sterols, but obtain them from their diet (Ritter 2010).

Other nutrients, including vitamins and minerals show high variability according to many factors, including insect species, metamorphic stage, diet, processing and preparation, habitat, and climate (Bukkens 1997; Chen, Feng et al. 2009; Verkerk et al., 2007). Consequently, levels

of nutrients can be modified during the feed chain according to the goals sought (Pennino et al., 1991). In general, most insects are an excellent source of iron and zinc, which are the cause of human nutritional deficiencies in developing countries (van Huis et al., 2013). In the case of iron, some species like mopane caterpillar or locusts (*Locusta migratoria*) have even a higher content of iron than beef, which is already known as a high source of iron compared to the other conventional meat group (Bukkens, 2005). Iron deficiency could be avoided with a well-balanced diet, yet it is one of the most common nutritional disorders (anaemia) in developing countries (van Huis et al., 2013). Zinc deficiency is another core issue for the health of child and pregnant women, leading to growth retardation, delayed sexual and bone maturation, alopecia, diarrhoea and defects in the immune system (FAO/WHO, 2001). It is interesting to know that an edible insect like the Palm weevil larva (*Rhynchophorus phoenicis*) contain more than twice the level of zinc compared to that of beef (Bukkens, 2005).

Such a high nutritional value of insects should be taken into account for not only for animal intended feedstuffs but also for food that can entirely or partially replace conventional meats in human diet. A trend in a high level of consumption of meat-based proteins has been recognized as a significant cause for the increased prevalence of non-communicable diseases, such as cancer, especially in Western countries (Alexander et al., 2010, Alexander et al., 2010a; Corpet, 2011; Magalhaes et al., 2012). Good nutritional guidelines aim to promote a partial substitution of meat-based proteins by other protein sources, such as fish or plant proteins (Gerbens-Leenes and others 2010; Aiking 2011). Besides high nutritional value of insects, there are other reasons to encourage the consumption of edible insects rather than fish and plant proteins.

- **Fish** consumption is known to offer many health benefits owing to a high level of omega 3 polyunsaturated fatty acids, yet one fish and shellfish tend to concentrate methylmercury (MeHg), a well-known environmental neurotoxin, affecting in particular young children and pregnant women (Mahaffey et al., 2011);
- In the case of **plant protein sources**, such as grains and pulses, there might be a potential risk of fungal contamination and mycotoxin-poisoning. Intake of mouldy grain and pulses with mycotoxins may cause adverse effects in the body, including mutagenicity, carcinogenicity and organ toxicity (Peer & Linsell, 1973; Gelderblom et al., 1988; Jay, 1991).

To continue with partial substitution of meat by insects in human diet, edible insects deserve to be compared to conventional meats. Sirimungkararat and others (2008) concluded that 100g of eri silkworm (*Samia ricini* D.) and mulberry silkworm (*Bombyx mori* L.) have a similar energy content compared to the same amount of fresh pork meat. Although amino acid contents and their compositions differ from one insect species to another, the total protein content of many insect species may be equivalent or even higher than that of certain conventional meats (Bukkens 1997; Ramos-Elorduy 1997; Srivastava, Babu et al., 2009). Contrary to the high PUFA/SFA ratio of in edible insects, beef and pork contain very few PUFAs compared to far more SFAs (DeFoliart 1991). In terms of a nutritional aspect, edible insects are as efficient food material as conventional meats and provide more health benefits.

3.3 Chitin and chitosan

Chitin is a major component of cuticles or exoskeleton in insects. Concerning health hazards related to insect consumption, chitin is described as a causative agent of allergic and physical hazards with anti-nutritional properties (Veldkamp et al., 2012). Chitin, is in fact an important source of fibre, typical to arthropods, but also commonly found in fungi (van Huis et al., 2013). Chitosan is a derived, de-acetylated chitin after an alkali treatment which gives a more soluble product analogue while chitin has a low digestibility (James & Nation, 2015; Liu et al., 2012). Chitin and chitosan have been known for their biological properties, which attract a great attention for various industrial applications (Liu et al., 2007). They are non-antigenic and non-toxic, bio-degradable and bio-compatible (Shahidi & Abuzaytoun. 2005; Khor & Lim, 2003). The immunological effects of chitin have recently been recognized (Lee et al., 2008). According to Lee and other (2008), chitin has complex and size-dependent effects on innate and adaptive immune responses as inducing non-specific host resistance against microbial infections (van Huis et al., 2013). Anti-biotics are commonly used in livestock to prevent them from microbial infections. Therefore, chitin as an immune-stimulator, and may reduce the frequent use of anti-biotics in livestock.

3.4 Performance studies with insects as feed ingredient

3.4.1 Aquaculture species

Currently, about 10% of the global fish production goes to fishmeal that is then mainly used to feed other fishes through aquaculture (FAO, 2012). The price of fishmeal has been increased by three-folds for the last decade due to increased demands for fishmeal (Vrij, 2013). Aquaculture is also one of the fastest growing sector, in relation with animal intended food

production to provide animal proteins, responding to a growing world population and more demanding consumers (van Huis et al., 2013).

Many trials to replace fishmeal by insect meal concluded that a **minimum** 25 % of the fishmeal may be replaced by insect meal without lowering feed conversion rate (Sheppard et al., 2007). St Hilaire (2007) also concluded that 15 to 25% of the fishmeal can be replaced by larvae of black soldier flies (*Hermetia Illucense*) and house flies (*Musca Domestica*) in the diet of trouts without generating negative effects on feed conversion rate. In Africa, Alegbeleye (2011) and Jabir and others (2012) set many trials so as to find the correct proportion of fishmeal replacement with grasshopper meal (*Zonocerus variegatus* L.) and superworm meal (*Zophobas morio*) in the normal diet of the respectively juvenile African catfish and Nile tilapia (*Oreochromis niloticus*). About 25 % of replacement was suggested as it is the average amount causing no adverse effects on growth rate in the two studies. Meanwhile, Ogunji and others (2008) have demonstrated more detailed effects on fishes resulting from an increasing ratio of insect meal up to 100 % in the normal diet of different captured fishes. In a study by Ogunji and others (2008) on replacement of fishmeal by Magmeal™ (maggot powder mainly consisting of housefly larvae) (*Musca Domestica*), physiological stress factors were also analysed in order to investigate how the fish reacts to the maggot powder, whose fat content (~20 %) is higher than that of fishmeal (~8 %) in the diet of *Oreochromis niloticus* fingerlings. There were no remarkable differences in growth and feed conversion rate with up to 68 % of replacement by Magmeal™ and this level did not cause any stressful conditions which may be explained by a decreased growth rate, decreased haematocrit and haemoglobin values, and increased blood glucose and plasma cortisol concentrations (Ogunji et al., 2008). Based on studies investigating the appropriate ratio of replacement, a level of replacement of 25 % by insect meal gave the best performances without physiological symptoms showing stress whereas a higher replacement than 25 % brought lower growth and feed conversion rate (Sogbesan, 2006). The exact cause is still not known, but there are possible explanations, such as a higher fat or chitin level, or the lack of certain nutrients, including amino acids, vitamin and minerals.

When insect meal is aimed to aquaculture, there are two typical features of insect meal that should be discussed.

The first point is how well aquaculture species digest chitin present in their insect based diet. Insect proteins are known to be highly digestible (between 77% and 98%) even if the presence

of chitin in their exoskeleton lowers the value of digestibility (Ramos-Elorduy et al., 1997). Chitin is a structural poly-saccharide with one extra amine (NH₂) group, to be found in the exoskeleton of insects as well as of crabs, shrimps and other shellfishes (Vrij, 2013). Chitin is more difficult to digest than other structural poly-saccharides and un-digested chitin can function as a functional fibre which increases viscosity in the intestines and that fact reproducibly lowers blood cholesterol level (Vrij, 2013). Chitosan, a derived form after the removing of acetyl groups from chitin, has the same effect on improving blood cholesterol level by reducing the low-density lipoprotein oxidation (Bays et al., 2013). On the other side, chitin and chitosan are known to bind along the intestinal linings and forming gel-like substances with lipids that finally capture some vitamins and minerals before they are absorbed throughout the GI tract (ANSE, 2015). In fact, the effects of chitin or chitosan present in insect meal intended to aquaculture are not fully understood. Chitin removal may increase the digestibility of insect meal in animals and humans. With current technology, chitin cannot be extracted alone from cuticles of insects by any solvent, but it can be left behind after all other components are removed (James & Nation, 2015). For instance, alkali treatment can transform chitin into less acetylated chitosan, which is more transparent and flexible and also more soluble and digestible (James & Nation, 2015; Liu et al., 2012). Biological properties of chitosan have also attracted many researchers so as to explore further application of that product into the agricultural, industrial and medicinal fields (Liu et al, 2007). In addition, researches on the positive effects of chitin or chitosan on immune stimulation are currently being carried out in connection with the development of insect food and feedstuff (Vrij, 2013).

Another crucial point to consider about the insect meal intended for aquaculture is to maintain a consistent and normal taste of the fishes even after they are fed with feed different to usual. The taste of fish meat varies according to the different species, but more importantly, it can be altered within the same species depending on other external factors, including the salinity of the water where fishes are caught, or what kind of food they eat, or in which conditions they are stored or even prepared after capture (Vrij, 2013). Among free amino acids, glycine and glutamate are known to give fish a typical fishy and savory taste (McGee, 2007). Sealey (2011) demonstrated that there was still a consistent taste in fish meat fed with Black soldier flies, compared to the control group with had a conventional diet.

3.4.2 Poultry species

Insects are parts of the a natural diet in the poultry species, as in fact all birds kept in a free-range system are often exposed to consuming insects from surroundings. Ravindran and Blair (1993) replaced poultry soybean meal with the Black soldier flies (*Hermetia illucens*) and the Housefly pupae (*Musca domestica*) that were reared on chicken manure. More recent experiments demonstrated that a replacement with 5 to 6% of BSF meal resulted in similar performance with broiler chickens fed with either fishmeal or soybean meal. The same replacement showed similar growth rates compared to fishmeal in the starter period and the same results came also with soy replacement in the grower phase (Veldkamp et al., 2012). Recent studies conducted many trials for broilers with housefly maggot meal. 25% of maggot meal in the diet yielded better live weights, feed intake and daily gain in comparison with the same amount of fishmeal in diet. Other layer trials showed that maggot meal replacement over meat and bone meal increased egg production as well as hatchability (Veldkamp et al., 2012).

Insect meal as protein supplement, or replacement of fishmeal and soybean meal are not a new method and have already been practised at local farms in developing countries. Silkworm pupae, as by-products of silk manufacturing, could also replace an entire portion of fishmeal in the diet of layers (Joshi et al., 1979; Khatun et al., 2005), and it resulted from that type of diet that the growth rate, egg production and profitability almost linearly increased up to 6% of dietary levels (Khatun et al., 2005). Indeed, many related studies and experimental trials have been published by scholars and researchers issued from the Indian continent and South East Asian countries, where the poultry industry has been one of the fastest growing agro-businesses in the last decades (Veldkamp et al., 2012). However, feeding poultry with an expensive maize as well as soybean meal or fishmeal whose price has nearly doubled in the last 10 years, financially threatened local farmers in developing countries. A year-round warm climate and high humidity in such countries favours optimal conditions for rearing insect meal, and moreover the recycling of organic wastes satisfies both financial and ecological levels, as chicken-manure feeding insects and by-products of sericulture are feeding directly or indirectly the local poultry species.

Elorduy and others (2002) demonstrated how insects can up-grade a low-grade bio-waste into a high quality protein. In the same experiment, mealworms (*Tenebrio molitor*), which were reared on low-nutritive waste products, became a high quality protein meal for broiler chickens. Similar results of high performances in growth and egg production were obtained on trials with the House cricket (*Acheta domesticus*), the lesser mealworm (*Alphitobius diaperinus*) and the

Mormon cricket (*Anabrus simplex*), instead of both fish and soybean meals (van Huis et al., 2013).

3.5 Environmental impacts of edible insects

It is inevitable to link global food and feed security in order to feed a continuously growing world's population with more demanding consumers (van Huis et al., 2013). Livestock production is estimated to more than double by 2050 to meet human needs (van Huis et al., 2013). A high demand for animal protein sources has the consequences to put heavy pressures on limited sources, such as energy, land, oceans, and water, from which such food and feed sources are produced (van Huis et al., 2013). Large-scale livestock industry with more intensive producing systems has facilitated a high productivity, giving global feed security and economic viability in a short term, but unfortunately have led to huge environmental cost at the same time (Tilman et al., 2002; Fiala, 2008). If agricultural production remains using the same development systems, without searching environmentally sustainable alternatives to food and feed sources, it will be more and more difficult to mitigate the current heavy pressure on livestock and fish production. There will then be not only deforestation and environmental degradations, but also climate changes are set to accelerate (Sachs, 2010).

Besides the high nutritional value of edible insects, their environmental sustainability has been inspiring many people to re-consider them as the best sustainable food and feed sources. It is thus crucial to investigate their ecological footprint throughout the procedures of food and feed chain (Belluco et al., 2013).

3.5.1 Feed conversion rate

Compared to other alternative protein sources, consumption of edible insects has positive impacts on the environment. Firstly, insects have a high feed to meat conversion rate compared to other conventional meats (van Huis et al., 2013). Livestock and fish produce high-quality animal protein from lower-quality protein food sources originated from plants or small forage fishes or krill meal (small shrimp and crayfish or lobster species) (Vrij, 2013). For these animals, the feed conversion rate largely depends on both the class of animal concerned and the types of production practice (van Huis et al., 2013). According to calculation by Pimentel and Pimentel (2003), about 6 kg of plant protein are needed to produce 1 kg of high-quality animal protein

in livestock and fish. The US production system gives the following figures: 1 kg of live animal weight requires the following amount of feed: 2.5 kg for chicken, 5 kg for pork and 10 kg for beef, whereas the production of 1 kg of live insect weight of crickets requires about 1.7 kg of feed (Smil, 2002; Collavo et al., 2005). Moreover, if these figures are calculated from the edible weight of livestock, fish and insect, and not from the live weight, the advantage of eating insects become even greater since 80 % of one cricket is edible and digestible compared to chicken and pigs (55 %), and cattle (40 %) (Nakagaki & DeFoliart, 1991). Therefore, crickets are 2 times more efficient in converting feed than chicken, 4 times more efficient than pork and 12 times more efficient than beef (van Huis et al., 2013). At this stage, it is important to recall that insects do not need food to maintain their body temperature as they are cold-blooded animals (van Huis et al., 2013).

3.5.3 Recycling of organic wastes and bio-degradation

Among edible insect species, the black soldier fly (*Hermetica illucens*), the common housefly (*Musca domestica*) and the yellow mealworm (*Tenebrio molitor*) can be reared on organic side streams, such as manure, pig slurry and compost (van Huis et al., 2013). Thanks to their capability of bio-transforming organic wastes into bio-fertiliser, the application of such edible insects is expected to complete the economic and environmental cycle in the food and feed chain (Veldkamp et al., 2012). This chain starts with “processed” organic side-streams or bio-wastes where insects are reared in a sustainable way. These reared insects are processed into animal feed and then, the chain finally ends as feed ingredients used by aquaculture, poultry and pig rearing sectors. One of the key edible insects, crickets are usually fed with high-quality feed, yet the partial substitution with “*processed*” organic-wastes may allow the cricket-farming to be more profitable (Offenberg, 2011). Agricultural wastes and animal manure are one of the main causes inducing huge environmental costs (Belluco et al., 2013). It contaminates surface and ground water with pathogens and toxins, and potentially involves the emission of ammonia and the corresponding effects on the acidification of the ecosystem (ANSE, 2015; Tilman et al., 2002; Thorne, 2007). Therefore, the use of insects for their action of bio-degradability on organic wastes along the food and feed chain is a very efficient way to decrease organic pollution worldwide. Moreover, partial or entire substitution of insect meal in crop-based proteins will reduce deforestation as well (Belluco et al., 2013). However, there are still doubts relative to the safety of rearing edible insects on organic-wastes due to unknown risks of pathogens and contaminants that can be picked up and transmitted to the surroundings via flies.

This is why the rearing of insects on organic side streams is not currently permitted by the EU food and feed legislations.

“*Ecodiptera project*”, co-financed by the European program LIFE was launched in 2004 to reduce animal wastes, especially pig manure across the EU (van Huis et al., 2013). Larval flies are placed in solid manure separated from the urine as to bio-degrade and transform it into bio-fertilisers and protein rich foods as they grow into mature larvae and pupae. In Slovakia, a pilot plant for bio-degradation of pig slurry has been implemented with the development of methods for the maintenance of fly colonies under optimal conditions (Veldkamp et al., 2012).

Newton and others (2005) developed different types of swine systems to carry out the bio-degradation of pig manure using the larvae culture of Black soldier fly (*Hermetia illucens*) in the US Animal and Poultry Waste Management Center. Two types of swine systems were proposed, in which the culture of *Hermetia* larvae has been placed either right beneath pigs or away from pigs. Both systems allow bio-degradation in fully enclosed buildings at a temperature ranging from 27.5 to 37.5 °C. Some of the larvae were saved to support the adult soldier fly colony and their eggs were collected to maintain the larval densities in the cultures (Veldkamp et al., 2012). The remaining larvae were directly dried and processed for feed preparation or were let to develop into pupae as another feed preparation. Newton and others (2005) concluded that 0.214 kg/pig/day of larvae were collected for treatment 1 and 0.153 kg/pig/day ($P < .03$) for treatment 2. It was estimated to yield 64,000 kg of larvae annually for a pig house of 1000 individuals, 2.5 times per year. Still and despite a great profit in terms of economic and ecological level, there was a difficulty in managing a warm environment favourable for oviposition. Particularly in temperate regions, energy consuming for maintaining the internal temperature of a building between 27.5 and 37.5 °C throughout the year is too costly (Belluco et al., 2013).

With respect to potential risks of pathogens or contaminants derived from animal manure, I would like to recommend to have the rearing space for the adult fly colonies separated and away from the culture larvae where the “processed” solid manure is continuously provided, this in order to prevent the adult colonies from picking up pathogens and contaminants and transmit them to the surroundings.

Agriprotein Technologies, a new industry company producing insect-based proteins (Magma™) and oil (Magoil™) from nutrient recycling, has established its pilot plant near Cape Town, South Africa. The production chain starts with rearing stock flies in sterile cages

with over 750,000 flies per cage, and weekly about 750 to 1000 eggs laid per one single female fly hatch into larvae which will be fed with human faeces and animal blood from abattoirs (Veldkamp et al., 2012). According to Agriprotein Technologies, these larvae are usually harvested just before the pupae stage and then dried on a fluidised bed dryer before packaging in the form of flakes. And the Magmeal™ protein contains 9 essential amino acids with high content of Cysteine, Lysine, Methionine, Threonine, and Tryptophan.

3.5.3 Life cycle assessment

In order to investigate the ecological footprint of insects, several parameters, such as Life Cycle Assessment (LCA), greenhouse gas (GHG) production or GWP (Global Warming Potential), fossil energy use (EU), land use (LU) and finally water use (WU), are quantified during the insect food or feed production chain (van Zanten et al., 2014). The LCA aims to assess the environmental impacts during each stage of a product's life (van Huis et al., 2013), which, in the case of insects, from rearing up to consumers as food, feed and fertiliser and it is governed by the standards and guidelines of ISO 14040 and ISO 14044 (ANSE, 2015). The current livestock sector is responsible for about 15 % of the total emission of anthropogenic greenhouse gas (GHG) and should be considered as one of the largest contributors to global warming (Steinfeld et al., 2006; Steinfeld, 2012; Pan et al., 2011; Godfray et al., 2011). In addition, the livestock sector occupies about 70 % of the total agricultural land (Foley et al., 2011). Global production of bovine meat and milk may be responsible for respectively 41% and 20% of the total emission produced by farms, and pig or poultry production (including egg production) may account for 9% and 8%, respectively (Gerber et al., 2013). So, the LCA of insects to evaluate its sustainability encompasses such parameters to compare with the ones of other conventional protein sources, such as fish, poultry, pork and beef (van Zanten et al., 2014; Veldkamp et al., 2012). The global warming potential (GWP) measures the amount of greenhouse gas emission including carbon dioxide (CO₂), ammonia (NH₃), methane (CH₄) and nitrous oxide (N₂O) production, associated with average daily gain (ADG) as a measure of feed conversion efficiency (Oonincx & de Boer, 2012).

Oonincx and de Boer (2012) demonstrated the LCA of 5 edible insect species via three parameters, which are the GHG production, in other words, GWP (Global Warming Potential), energy use (EU) and land use (LU) in comparison with poultry, pig and beef throughout the entire sectors of the production chain. In terms of GWP, crickets, mealworm larvae and locusts

emit less GHG by a factor of 100 than pigs and cattle (Oonincx et al., 2010). Among 5 insect species, cockroaches, scarab beetles and termites produce methane originated from bacterial fermentation by Methanobacteriaceae in their hindgut (Hackstein and Stumm, 1994; Egert et al., 2003). It thus implicates that a careful selection of species, which do not have Methanobacteriaceae in hindgut, will allow to reduce the potential GHG emission. In the light of CO₂ and N₂O emission related to insects, it may be resulted from processing and transport of feed, and the figures of NH₃ emission was also lower than those of poultry, pork and beef (De Vries & de Boer, 2010). As it is mentioned previously, insects generally have a high feed conversion rate thereby they do not need much food compared to the conventional livestock. Moreover, the drinking water supply is not necessary as they have enough for their physiological needs through the water already present in their food. Dalgaard and others (2007) investigated two of three parameters (GWP and LU) in comparison between mealworms (*Tenebrio molitor*) and soybean production for animal feedstuff. The results indicate that the mealworm production is less ecological than soybean meal production for animal feed, but still better than conventional animal production. However, there was a missing parameter, the land use area, exploited largely for soybean cultivation and some livestock, like cattle. In fact, for every ha of land required to produce mealworm proteins, 2.5 ha for a similar quantity of milk protein, 2 to 3.5 ha for a similar quantity of pork or chicken protein, and 10 ha for beef protein (van Huis et al., 2013). There are no accurate data for the land use area in order to produce the same amount of soybean meal. Nonetheless, it cannot be simply concluded that mealworm production is less environmentally friendly compared to soybean meal if the land use area was not considered as one of parameters in the life cycle assessment. At the International Conference on Life Cycle Assessment in 2014, Van Zanten and others (2014) demonstrated a comparison of global warming potential (GWP), energy use (EU) and land use (LU) of larvae meal, fishmeal and soybean meal (SBM) calculated by ton of dry matter feed. The production of larvae meal and fishmeal results in high EU, which affects the GWP. However, the EU and GWP of fishmeal indicated an almost double value as those for larval meal production. The LU in larval meal and fish meal recorded almost none while soybean meal production is remarkably land-intensive (see Figure 3).

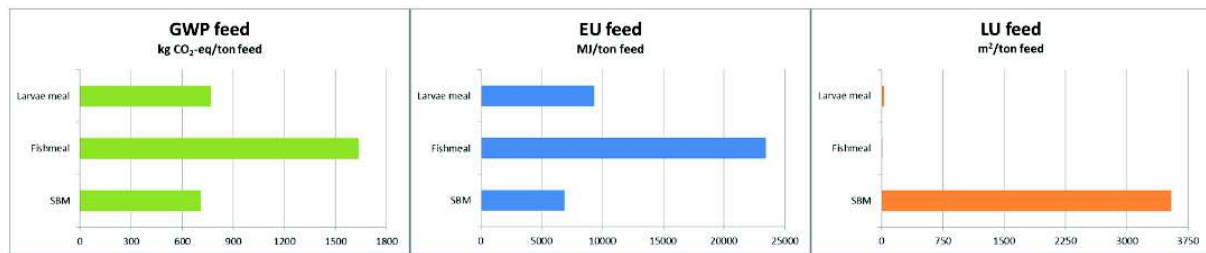


Figure 3. Comparison of global warming potential (GWP), energy use (EU) and land use (LU) of larvae meal, fishmeal and soybean meal (SBM) based on ton dry matter feed (Zan Vanten et al., 2014)

As for fossil energy use, mealworms (*Tenebrio molitor*) have almost an identical level of EU compared to cattle for the same amount of production. Besides, production of poultry, pigs and milk require even lower EU than *T. molitor* (Ooninx & de Boer, 2012; Veldkamp et al., 2012). Since insects are poikilothermic, the fact that insects cannot regulate their body temperature causes high energy consumption in temperate regions, such as European countries (Belluco et al., 2013). The optimal development of insects then requires a thermal comfort zone. For example, a condition with around 28°C and 70% of relative humidity altogether need to be satisfied to rear *T. molitor* (ANSE, 2015; Li et al., 2013). Even though insects have a high feed conversion as well as a lower need for land and water use compared to other warm-blooded animals for conventional meat production, rearing insects still requires high energy consumption to maintain the optimal temperature and humidity for their optimal development, especially in temperate thermal regions.

4. Health hazards and feed safety

4.1. Chemical hazards

When an issue of chemical risk caused by insects is emerged, most of people come up with ideas of pesticides and how pesticides residues affect on humans. Indeed, when my personal inquiry about “insects and chemical hazards” or “insects and toxic substances” was launched in the web-searching engines, there were a great amount of data informing about more efficient performance of new pesticides on crop pests, rather than intrinsic chemical substances produced by insects themselves. It clearly indicates that research has rather concentrated on “how to eradicate pest insects” than “how to exploit them by understanding chemical mechanisms of each species of insects”.

Potential health hazards caused by insects, especially their chemical substances, largely depend on insects themselves, including species, habitat, diet and other environmental factors. As not all insect species are edible, there are certain periods in life cycle when they may or may not be edible (ANSE, 2015). Edible mealworms can be eaten only at their larval stage whereas ants of several varieties be eaten throughout entire stages from eggs to adults in the different parts of the world (van Huis et al., 2013). It is not only because certain stages of life cycle are particularly unpalatable than other stages, but also ingestion of edible insects in certain developmental stages may also cause potential health hazards to consumers.

Many communities all over the world own such a long history or culture of consuming insects and they also have developed at the same time their own way of cooking insects as a part of culinary art. On the other side, they have noticed that some chemical substances can lose their toxic properties after various cooking processes (Berenbaum, 1993). Formic acid, the simplest carboxylic acid, is an example of endocrine venom which was found in nature by distillation of ant body (Hoffman, 2010). It has a relatively low toxicity with an LD50 of 1.8 g/kg (per oral) in mice and thereby is being used as food additive, but its concentrated acid can be corrosive to the skin. Before edible ants are consumed, formic acid of ants are removed by boiling or roasting (Reutemann & Kieczka, 2002). Nevertheless, some insects always remain inedible even after such processing, including boiling, frying, steaming, and drying, mostly for chemical substances like certain pollutants, heavy metals, residues of insecticides and veterinary medicines.

4.1.1 Classification by glandular and non-glandular origin

The first category of chemical hazards caused by insects is insect-synthesized or accumulated toxic or noxious substances. Toxic substances can be manufactured by insects themselves as defence or repellent mechanisms against predatory insects or animals. According to the origin of synthesis or secretion, toxins are distinguished between endocrine and non-glandular origin. As an example of endocrine originated toxin, bombardier beetles (Carabidae family) emit the compounds of hydrogen peroxide and hydroquinone in the form of 100°C ejection (Eisner, 1970). Some insects also warn their predators by showing certain colours or colour patterns (Zagobelny et al., 2009).

Bright coloured day-flying moths, *Zygaena filipendulae* (Lepidoptera Order) are known for liberating toxic hydrogen cyanide that is sequestered from food plants, *Lotus corniculatus* (Zagobelny & Møller, 2011), although they de novo biosynthesize from the amino acids valine and isoleucine respectively (Jensen et al., 2011). Such phytophagous insects can accumulate the same toxic properties as their host plants (ANSE, 2015; Barenbaum, 1993). It indicates that insects feeding on edible plants without toxic substances can be considered as safe (Holt, 2007). The food plant, *Lotus corniculatus* is not particularly poisonous to humans due to relatively little dose. However, potential toxicity of cyanogenic glycosides can manifest itself due to fast enzymatic degradation (Frakes et al., 1985; Frakes et al., 1986). Hence, cyanoglycosides even with a little dose can be lethal to humans despite cyanide is detoxified by the enzyme rhodanese in human body (NZ food safety authority). Both *Lotus corniculatus* and its consumer, *Z. filipendulae* are considered not edible.

4.1.2 Classification by phanerotoxic and cryptotoxic insects

Another classification is made between external venomous phanerotoxic and cryptotoxic insects, and in the case of the latter one, the toxic substance only appears when the cryptotoxic insect is consumed (ANSE, 2015; Belluco et al., 2013). Phanerotoxic insects include piercing (Hemiptera, including true bugs and cochineals) and stinging (Hymenoptera including bees, wasps and ants) species which inoculate venomous substances or species of Lepidoptera with urticating bristles, followed by inflammatory reaction (van Huis et al., 2013). In Japan, there was one case when bristles of larvae of *Trogoderma* spp. caused ulcerative colitis in a 4 year-old boy by ingestion of grain infested with *Trogoderma* spp. (Okumura, 1967). It is assumed

that venom-inoculation as well as urticating parts typical to phanerotoxins may cause this incidence.

Cryptotoxic insects are able to synthesize or store noxious substances that only appear when they are ingested (ANSE, 2015). Among edible insects, lepidoptera are well known to bioaccumulate toxic substances very easily (Wagrobelny et al., 2004). Cyanogenic compounds are often present in Coleoptera and Lepidoptera (Blum, 1994). Metabolic steroids, such as dihydrotestosterone or testosterone, can be found in beetles, whose clinical symptoms manifest as growth retardation, jaundice and liver cancer, and hypo-fertility and masculinisation in females (Belluco, 2013).

Moreover, some phytophagous insects may be potential cryptotoxic insects since they accumulate toxic substances from plant food. On the other hand, they can also detoxify, bioaccumulate, and excrete those toxins in order to avoid intoxication themselves (ANSE, 2015). In addition, there are variations in the accumulation of toxic substances within the same species, according to life cycle, developmental stage and physiological status (Bennett and Wallsgrove, 1994). Certain phyto-toxins are present as secondary metabolites, such as alkaloids, cardenolides, cucurbitacins, glucosinolates, or phenolic or cyanogenic compounds (Bennett & Wallsgrove 1994; Nishida 2002). Among them, quinones or phenolic compounds are widely distributed in plants and as well as in insects of the Family Tenebrionidae (Belluco et al., 2013). *Ulomoides dermestoides* (Order Coleoptera), Tenebrionid beetles have been applied in Chinese and Japanese folk medicines for the treatment of respiratory disorders (Santos et al., 2010). Later on, they became popularly reared by Central and South Americans for the treatment of asthma, Parkinson's disease, diabetes, arthritis, HIV and especially cancer (Santos et al., 2010). Although some researchers proved the great relevance of the aqueous extract of *U. dermestoides* as anti-inflammatory agent, the blend of their secretion as defence mechanism contains benzoquinones, which are the family of quinones (Santos et al., 2010). According to several studies demonstrating the effect of defence compounds of *U. dermestoides*, benzoquinone-containing secretion can be cytotoxic against human lung carcinoma epithelial cell line A-549 (Crespo et al., 2011). Moreover, it reduced cell viability and induced DNA damage in human cells (Crespo et al., 2011). Some researchers also concluded that the human DNA damage by benzoquinone may lead to a cancer only by contacting with benzoquinone (Belluco et al., 2013).

4.1.3 Edible insects: origins of substrates in their diet

Recently, a huge attention has been paid on the EU legislations covering the safe use of insects as food and feed sources. Prior to the re-framework of regulations concerning edible insects, it is necessary to assess potential health risks that can be caused by consuming insects. In particular with insect meal directed to conventional livestock, selection of safe substrate in the diet of edible insects is another crucial task to fulfil during a re-assessment of health risks. It is because any toxic substances accumulated from their diet may directly pass onto future consumers. According to food and feed legislations, edible insects, especially for livestock consumption, are not allowed to feed on organic side streams. For instance, animal manure is part of the natural diet in housefly larvae, which is one of the key edible insects for both animals and humans. Manure is classified in Category 2 materials and only Category 3 materials can be used for insects intended for livestock consumption. The Dutch insect farm, Kreca produces 12 different species of insects, intended for pet food (95%) and human consumption (5%). They use only corn meal and goat meal for those species. However, not all insects have naturally vegetable-based diet and not all phytophagous insects have the same mechanism of physiology for all plant foods. In order to comply the strict regulations of the EU, insects have to be fed with 100% vegetable-based diet. However, if the 100% vegetable-based diet does not fit them physiologically, some of them may bio-accumulate secondary metabolites from their diet. It is thus necessary to continue researches or studies on toxic substances not only from insects but also from their food plants. Moreover an intensive assessment in the safety of rearing insects on organic side streams and corresponding sanitary controls during the food and feed chain should be provided for the re-framework of the EU regulations as well as industrialization of the edible insect market.

4.1.4 Anti-nutritional substances in insects

Insects also have anti-nutritional properties like many other edible plants, such as grains and pulses. Belluco and others (2013) suggested that anti-nutritional substances in edible insects may become a serious health hazards to consumers, especially for those with poor diet in specific nutrients, such as vitamins and minerals (Belluco et al., 2013). Almost nothing is known in terms of anti-nutritional properties of insects since they have never been regarded as food or feed in developed countries, including the EU and North America. According to ANSE (2015), there are 5 anti-nutritional substances identified among insects, including phytic acid,

oxalates, hydrocyanic acid, tannins and thiaminase. There is very little information concerning anti-nutritional factors present in insects, except for thiaminase (ANSE, 2015).

a) Thiaminase

Thiaminase is a catalytic enzyme responsible for avitaminosis syndrome causing brain damages and neurological disorders, including ataxia (ANSE, 2015). It metabolizes and breaks down Thiamine, vitamin B1 into two molecules (Fujita et al., 1952). Thiamine-deficiency in non-ruminant animals manifests variously as anorexia, impaired reproduction, cardiac enlargement and muscular weakness leading to ataxia (Kraft et al., 2014). In humans, the disease with the same aetiology and similar symptoms is named “beri-beri” (Nguyen-Khoa, 2015).

There are at least two types of thiamine-degrading enzymes, thiaminase 1 and 2 (Jenkin et al., 2007). Thiaminase 1 is the most common form, secreted by ferns, fishes, freshwater mussels, protozoa and bacteria, whereas thiaminase 2 is present in certain bacteria (Kraft et al., 2014). Thiaminase is well known to be present in raw flesh and the viscera of some fishes and even a few strains of bacteria, such as *Bacillus thiaminolyticus*, *B. neurinolyticus*, *B. subtilis* and *Clostridium Botulinum* (Boś & Kozik, 2000; Wittliff & Airth, 1968; Nakatsuka et al., 1988). Its activity is also present in most parts of plants, but restricted to such Pteridophytes (Evans, 1976; McCleary & Chick BF, 1977). It was first reported that fur producing foxes showed ataxic neuropathy after they were fed by raw entrails of river fishes in 1941 (Woolley, 1941). Then, thiaminase-containing plants, including horsetail (genus *Equisetum*) and bracken fern (*Pteridium Aquilinum*) caused cerebrocortical necrosis and polioencephalomalasia in sheep and cows respectively (Evans, 1975; Ramos et al., 2003).

The first case of insect-thiaminase manifesting in human population was reported in Eastern Nigeria (Adamolekun, 1992). An acute, seasonal ataxic syndrome was implicated based on the facts that there has been traditional entomophagy of pupae of African silkworm (Adamolekun & Ibikunde 1994a; Wright & Morley 1958), *Anaphe venata* caterpillar as an important protein source in this region and this syndrome has been observed for more than 40 years during the rainy season between July and October when people particularly consumed *A. venata* (Adamolekun 1993a, Adamolekun et al. 1994). Furthermore, the administration of thiamine could relieve the severity of symptoms in a randomized, double-blind test (Adamolekun et al., 1994). Likewise, thiaminase deficiency usually responds well to the early diagnosis and treatment, such as intramuscular thiamine injections and removal of sources of thiaminase both at the same time (Nguyen-Khoa, 2015). Otherwise, thiaminase can be denatured or at least

reduced by heat treatment, such as cooking or boiling process (Nishimune et al., 2000). Indeed, Australia's sorigines used to cook Nardoo, a local fern whose thiaminase activity is up to one hundred times than that of a broken fern, in order to avoid thiamine-poisoning (Nguyen-Khoa, 2015). Nishimune and others (2000) preliminarily measured the thiaminase activity in Japanese silkworms (*Bombyx mori*) that actually are consumed as snack in South Korea. In Korea, silkworms are cooked in moderately salted soup. Although its thiaminase 1 activity was measured as less than one-third of that of *Anaphe* spp, the heat-cooking process should be maintained to reduce the level of thiaminase activity (Nishimune et al., 2000).

b) Phytic acid or phytate

Phytic acid, phytate in salt form, can be often found in hulls of cereals, grains and nuts which could be the main diet for many reared insects (ANSE, 2015). When phytic acid is present in food, the bio-availability of phosphorus in consumers decrease as there are forming iron-chelating complexes (Cohen, 2015). If we can reduce or remove phytic acid with certain processing steps, the absorption of iron and other trace minerals can be improved markedly (Cohen, 2015). Insects can accumulate phytic acid through their diet, especially based on legume seeds. Therefore, it is necessary to undergo certain processing, such as putting legume seeds in mild (0.5 %) bicarbonate sodium solution in order to reduce or remove phytic acid from their diet (El-Adawy, 2002). Phytic acid in soy products can be removed through fermentation by natural enzymes found in several microbial species, such as *Aspergillus* species (Fukushima, 1991). However, there is no literature informing of the intrinsic phytic acid or phytate in insects.

c) Oxalate

Oxalate, which is often present in healthy green leaves, including spinach and kale, interferes Ca absorption and bio-availability when sequestered in the form of Ca oxalate via ingestion (Korth, 2006). Oxalate is one of the defensive mechanisms in plants for deterring phytophagous insects by crystalizing oxalate in body tissues (Bidlack & Rodriguez, 2011). The crystallisation of oxalate in body tissues leads to an irritation on the digestive tract, blood circulation disorders and kidney damages when absorbed in large quantity (ANSE, 2015). Cooking process may reduce the level of oxalate in their plant foods (Bidlack & Rodriguez, 2011). Phytotoxins, such as oxalates, glycosides and goitrogens have been manipulated within plants to enhance the resistance against insects or developed into pesticidal products (Bidlack & Rodriguez, 2011). However, there is no literature informing the intrinsic oxalate present in insects.

d) Hydrogen cyanic acid

Hydrogen cyanic acid, or hydrocyanic acid is a solution of hydrogen cyanide in water, which is highly toxic and thereby used as poison and chemical weapons (Gail et al., 2005). It can be isolated from fruits with pits, such as cherries, apricots and as well as bitter almonds (ANSE, 2015). Some millipedes or certain insects, like burnet moths release hydrogen cyanide as repellent mechanism (Blum & Woodring, 1962). In the case of Millipedes (Class Diplopoda) they have never been so far considered as mini-livestock since most orders of millipedes are known for their chemical repellent (Pemberton, 2005; Paoletti & Dufour, 2005). However, their chemical substances have a great potential for deterring mosquitoes, plasmodium and other parasites (Enghoff et al., 2014). Burnet moths (*Zygaena filipendulae*), which are widely distributed throughout Europe, contain cyanide in their bodies and warn their predators with bright coloured body part or certain patterns of spots on their wings. They are known as poisonous at both larval and adult stages (Scriber, 1984).

e) Tannin

Tannins are the most abundant secondary metabolites widely distributed throughout plant kingdom as a defence mechanism against predation (Barbehenn, 2011). Tannins are distributed in 180 families of dicotyledons and 44 families of monocotyledons, commonly ranging from 5 to 10 % of tree leaves DM (Barbehenn, 2011; Mole, 1993). Tannins can be toxic as they precipitate proteins at a high dose (ANSE, 2015). Dietary tannin can reduce growth rate and fecundity of some phytophagous insects (Shultz, 1989). On the contrary, recent studies emphasize that tannin can be nutritionally positive with an appropriate dose (Barbehenn, 2011). Moreover, insects also have developed the ability of tolerating ingested tannins with own defence mechanisms in their guts, such as anti-oxidants or a protective peritrophic envelope lining on the midgut, or high pH surfactant (Barbehenn, 2011). Besides, tannins can be extracted by the means of many types of solvents, including water and acetone. Hot water is used commercially, otherwise acetone at a concentration of 70% can used in laboratories (Seigler, 1981). Tannin extractability depends on the different types of tannins at different concentrations (Seigler, 1981). Further research work remains needed especially on the effects of different types of tannins relative to different species of phytophagous insects that can be reared as mini-livestock, such as caterpillars and grasshoppers (Barbehenn, 2011).

4.1.5 Heavy metals, pesticides and residues of veterinary medicine

Insects accumulate undesirable substances found in their food or surrounding environment through ingestion of contaminated food and water for instance. A variety of sequestered phytotoxins and bio-accumulated metabolites from the plant diet of insects is an example of extrinsic undesirable substances present in insects. Besides certain phytotoxins and secondary metabolites, there are pesticides, heavy metals, persistent organic pollutants as well as veterinary medicine residues.

Bio-accumulation of residual pesticides or persistent organic pollutants in insects is poorly documented in scientific literature. There is only one study that investigated residual pesticides present in edible locust captured in the wild, Kuwait (Saeed et al., 1993). Local people have a tradition to consume wild locusts, so the health risk caused by consuming wild locusts was evaluated by extraction of residual pesticides from randomly captured locust of the study area, using gas chromatography (Saeed et al., 1993). The result addressed that a relatively high amount of phosphorus residues of Sumithion or Fenitrothion was present in the samples. Fenitrothion is a globally used, contact organophosphate insecticide on a variety of crops and fruit trees (Wang et al., 2012). In spite of regulation levels of Fenitrothion as well as residues in food and feed at both national and international level, chronic contact and ingestion of residues may give rise to serious health risks. Saeed and others (1993) extracted 760 µg/kg of Fenitrothion while its tolerance limit is known as 100 µg/kg. This study has a great implication on the importance of establishing a large scaled rearing system in which more strict controls have to be applied to pose no additional health hazards compared to the traditional way of capturing or farming insects. Moreover, those chemical compounds are generally eliminated by processing or treatments (ANSE, 2015).

Crickets or termites, as other soil-dwelling insects have a potential opportunity pass on heavy metals, pollutants and other contaminants taken from solid waste through the food web or pyramid of the ecosystem (Gaylor et al., 2012). Zhuang and others (2009) investigated the bio-accumulation of potential heavy metal content along the soil-plant-insect-chicken food chain. The results demonstrated that Cd (Cadmium) declines with increasing trophic level whereas levels of Zn (Zinc) and Cu (Copper) increase with plant food given to insect larvae, although these elements were effectively eliminated through the faeces of insects (Zhuang et al., 2009). As (Arsenic) was present in a moth consumed by aborigines of Australia (Green et al. 2001). Cd (Cadmium) was found in the larvae of *Tenebrio molitor* (Vijver et al. 2003) and Pb (Lead) in grilled crickets in Mexico (Handley et al. 2007). As insects are parts of natural diet in poultry,

they tend to accumulate these metal elements via the ingestion of contaminated insects when reared in the open-air. According to Zhuang and others (2009), chickens fed with Pb (Lead) accumulated in insect larvae indicated that the metal concentration is turned out to be higher in the liver and lower in the blood within the same individual. As for persistent organic pollutants accumulated in insects, Gaylor and others (2012) demonstrated the capacity of house crickets to bio-accumulate poly-brominated diphenyl ethers (PBDE), which are abundant in human spaces and vehicles, (in this study, directly found in polyurethane foams). PBDEs are organic compounds but resistant to environmental degradation (Ritter et al., 2007). Those persistent organic pollutants adversely affect a whole ecosystem, persist and remain accumulated in the environment with the possibility of passing through the food chain (Ritter et al., 2007). Invertebrate organisms, such as insects or arthropods can facilitate the trophic transfer of such pollutants to other organisms after breaking down complex organic matter, but without being absorbed across the gut (Gaylor et al., 2012). Throughout the entire insect food and feed chain, the methods, processes and equipment used to be carefully studied and selected since these factors can be responsible for the transfer of contaminants as food contact materials (Belluco et al., 2013).

Insects also bio-accumulate the residues of veterinary medicine via contaminated water and food with manure coming from medically treated animals. Floate (2007) claimed that the potential risk of endectocide residues on non-pest insects, such as dung-dwelling or feeding insects, including some threatened or endangered species, has been underestimated. Furthermore, if insects become industrialised as mini-livestock in the near future, the use of veterinary medicines will be inevitable like in other existing conventional livestock farms. At the same time, this is why there are very few researches on the potential effects of veterinary medicines in insects. In the case of treating bacterial or parasitic infections in livestock a certain period before slaughtering should be considered until applied medicines are metabolised and removed completely from their body. Cappelozza and others (2011) explored flacherie disease caused by *Enterococcus mundtii* in silkworms (*Bombyx mori*) and the treatment with a broad-spectrum antibiotic, chloramphenicol. Regardless aetiological factors, *Enterococcus mundtii* is also a human-associated microbiota, which may postulate the epidemiology in humans. Furthermore, they administered chloramphenicol through their food to silkworms and it was proved that chloramphenicol remained activated in the gastro-intestinal tract of these treated silkworms (Cappelozza et al., 2011). In addition, administered chloramphenicol is prohibited to use in farm animals according to EU Regulation No. 37/2010 2010. It is hardly possible to

treat insect individuals but the administration of some deworming or antibiotics is necessary in insect farms. Therefore, the use of veterinary medicine on insects should be studied by related scholars and researchers with setting up the re-frameworks of regulations including the usage of veterinary medicines in insects.

4.2. Chitin and Chitosan: as an anti-nutritional factor, or a cause of physical and allergenic hazards

ANSE (2015) included “chitin” of insects as an anti-nutritional factor that can cause intestinal constipation in monogastric animals without chitinase and may extend to intestinal obstruction in severe condition. Furthermore, chitin and chitosan can attach to the intestinal linings as binding lipids to form gel-like substances that finally capture some vitamins and minerals before they are absorbed throughout the GI tract (ANSE, 2015). In Java, Indonesia, several people had to undergo the surgical intervention due to the ingestion of large quantities of roasted scarab beetles (*Lepidiota* spp.) that led to a total constipation of the GI tract (Kuyten, 1960). Chitin, the second most abundant fibre or biopolymer, is commonly found in the exoskeleton of arthropods, including insects, crustaceae, arachnids and myriapods (millipeds, centipeds, and others) (Khor & Lim, 2007).

Besides their anti-nutritional properties, chitin of insects may be a causative agent of physical hazards although insects are not a classic vector of physical hazards in animals and humans, like other foreign materials that may be gained from the environment or re-contaminated in the middle of processing. However, it can cause such risks when hard, sharp and pronounced parts of insects, such as exoskeleton, rostrums and wings or elytra are ingested since edible insects are also eaten as a whole without any preparation nor removal of hard parts of the body (ANSE, 2015). Bouvier (1945) reported that in the Democratic Republic of the Congo, human consumption of grasshopper and locusts without removing their legs caused large spines of legs to be caught in the gut and only the surgical intervention could remove the legs from the gut. Therefore, the Federal Agency for the safety of the food chain (FASFC) advised to add a statement on the product label that the unpalatable parts, including legs and wings must be removed prior to consumption (ANSE, 2015). As a fact, some edible insects, such as the migratory locust are sold on the Dutch market with a statement advising to remove their legs and wings before ingesting them (van Huis et al., 2013).

Such potential risks can be solved with the processing or the grinding into the form of flour (ANSE, 2015). Otherwise, as chitin cannot be extracted from cuticles by any solvent, it can be left behind after all other components are removed. At first, an alkali treatment with KOH removes protein from the insect cuticles. At the same time, KOH also removes some of the acetyl groups from chitin and this process transforms chitin into less acetylated “chitosan” (James & Nation, 2015). Chitosan is transparent and flexible and a more soluble analogue of chitin (Liu et al., 2012). Chitin has low digestibility and assimilability in monogastric animals, yet it is still considered as a great source of fibre (van Huis et al., 2013).

In particular, chitosan attracts a great attention for variable applications in agriculture, industry and medicine thanks to their biological properties (Liu et al., 2007). Chitin and chitosan are non-antigenic, non-toxic and versatile biopolymers with remarkable biodegradability and biocompatibility (Shahidi & Abuzaytoun, 2005; Khor & Lim, 2003). Thus, when the concentrated protein extracts of insects will be accepted and then mass-produced in the near future, the derived industry in by-products of chitin and chitosan will grow as another higher value-added industry.

4.3. Allergens

Insect allergens have been discussed mainly on specific allergic reactions caused by insect stings or bites. Otherwise, they were mainly concerned by certain individuals in constant contact with insects, such as entomologists, agricultural or industrial workers (mostly with certain species of flies including mushroom flies and sewer flies, weevils, and larvae of flies and moths for fish baits), and laboratory workers (mostly with beetles, blowflies, cockroaches, crickets, flies, locusts, and moths) (Belluco et al., 2013). Recently, Cockroach allergy has been diagnosed worldwide along with urbanised, indoor life style. Now, time is upon us for the reevaluation of insects as food allergens since insects are being considered as the best alternative and sustainable protein sources for both animals and humans.

People with food allergy have an adverse, pleomorphic symptom in the body due to specific immune responses that occur reproducibly after exposure to certain foods (Sicherer & Sampson, 2014). The clinical symptoms of food allergy can be mild as urticaria but also severe as anaphylaxis that may cause a death (Belluco et al., 2013). Nonetheless, only few studies have been published concerning allergic reactions provoked by the ingestion of insects. Moreover,

the subject of edible insects involves cultural backgrounds and traditions in relation with geographical factors, which may result in various degrees of allergic risks (Belluco et al., 2013).

The risk of food allergy can be very predictable due to the concept of pan-allergens. Allergen is defined as “a protein or glycoprotein capable of binding immunoglobulin E (IgE)” according to the European Academy of Allergy and Clinical Immunology’s Immunotherapy Task Force (Alvarez-Cuesta et al., 2006), thereby “co-recognition” and “binding IgE by more than two proteins” would be applied to the term pan-allergens (Moreno-Aguilar, 2008). Pan-allergens are groups of evolutionarily conserved proteins found in various plant genera with a high degree of molecular homology (Miguères et al., 2014). Thus, each member of a pan-allergen family is co-recognized with another as it gives rise to “cross-reactivity” or “cross-allergy” (Belluco et al., 2013). Shellfishes, such as crayfish, lobster and shrimp, are widely consumed crustaceans and also known to induce allergic reactions in susceptible individuals (Ayuso, 2011). Such crustaceans have shown a high degree similarity in sequence of tropomyosin with other phyla of arthropods. Tropomyosin is a well-known pan-allergen or cross-sensitizing allergen, responsible for the immunological relationship between not only crustaceans but also other arthropods, such as arachnids and insects as well (Moreno-Aguilar, 2008; Belluco et al., 2013). Molluscs and helminths also share the similar allergens that are similar to those of insects (Barre et al., 2014). Therefore, it assumes that people allergic to lobster are likely allergic to other crustaceans as well as arachnids and insects (Leung et al., 1996; Reese et al., 1999). In fact, it explains that many allergic patients show allergic reactions upon contact to multiple allergens (Hauser et al, 2010). For example, some patients allergic to dust mites have been constantly and increasingly exposed to mites and became sensitive to certain seafood (Reese et al., 1999). People with seafood allergy are likely to have a tendency to be allergic to edible insects. Tropomyosin, a muscle-regulating actin-binding protein, can be found in both muscle and non-muscle cells of all species of vertebrates and invertebrates (Belluco et al., 2013). In addition, there are non-protein molecules that can induce IgE driven activities (ANSE, 2015). For example, cross-reactive carbohydrate determinants are commonly found in different kingdoms simultaneously, such as crustaceans, insect venoms, plant pollens and mites (Barre et al, 2014). In the light of allergic hazards by edible insects, most of the allergens correspond to ubiquitous proteins, in other words, pan-allergens that are classified into muscle proteins (actin, myosin, tropomyosin and troponin C), cellular proteins (tubulins), circulating proteins (hemocyanins and defensins) and proteins with enzymatic properties (alpha-amylase, arginine kinase, glutathione-S-transferase, triose-phosphate-isomerase and trypsin) (ANSE, 2015). As most

allergens are thermostable, it is essential to emphasize the importance of processing, such as boiling or roasting for removing allergic components (Phillips & Burkholder, 1995)

Virtually, any food ingredients can be allergic and this is not an issue confined only by humans, but also by other vertebrate animals (van Huis et al., 2013). With respect to insect-based meal, food allergies in the livestock have been poorly documented in scientific literature, yet the concept of “pan-allergen” cannot be ignored (ANSE, 2015). The cross-reactivity or cross-allergy allow to predict certain risks of allergic hazards in spite of poor knowledge, but the existence of pan-allergens may develop multiple sensitization even if it begins as minor allergens (Hauser, 2010). It is crucial to remark the possibility of cross-reactions between edible insects and other arthropods including crustaceans, mites, molluscs and even nematodes, therefore it also emphasizes conducting larger scaled studies and researches, especially in connection with molecule-based diagnostics (Hauser, 2010).

Even before edible insects became suggested as sustainable and alternative protein sources to solve the world’s food security, there were still several allergic phenomena following the accidental ingestion of insects. “Pancake syndrome” was characterized by severe allergic manifestations, including anaphylaxis in atopic patients, shortly after accidental ingestion of mite-contaminated wheat flour (Belluco et al., 2013). Pancake syndrome was observed frequently in tropical/subtropical environments (Sánchez-Borges et al., 2009). As many cases of Pancake syndrome were manifested during physical exercise, it is thus described as mite ingestion-associated, exercise-induced anaphylaxis (Sánchez-Borges et al., 2009). The meaning of “pancake” in this syndrome implicates that thermo-resistant allergens from cooked food could trigger allergic manifestations (Belluco et al., 2013). In the US, concerning such impurities in food, Food Defect Action Levels of Food and Drug Administration (FDA) restricts an average contamination level below 150 insect fragments per 100 g of wheat flour, which poses no health hazard (van Huis et al, 2013). In fact, it has been recommended to store flour in sealed or closed containers at a refrigerated temperature in order to inhibit proliferation of mites (Sánchez-Borges et al., 2009).

A recent study demonstrated 16 cases of allergic hypersensitivities, accompanied with asthma and anaphylaxis, due to the infestation of lentils with lentil pests, mainly *Bruchus lentis* in Spain (Armentia et al., 2006). Lentils are the most common edible pulse that involve allergic reactions, and it has been evaluated that the allergic symptoms were associated with the legume protein of lentils themselves or pest proteins. A skin prick test and an oral test with boiled, infested lentils were both performed on patients who had allergic hypersensitivities after the intake of

infested lentils, and both tests have turned out positive, proving that insect proteins can lead to IgE-mediated rhino-conjunctivitis and asthma in susceptible people (Armentia et al., 2006).

Another example of allergic manifestation is carmine, a biologically derived food colorant from the dried bodies of female cochineal insects (*Dactylopius coccus costa*) (Belluco et al., 2013). Although carmine is widely used as a natural dye in foods and cosmetic products, many people are not aware of the origin of this colorant (DiCello et al., 1999). It is not likely regarded as a potential cause of food allergy since the residual protein allergen in carmine is at a very low level (Taylor & Dormedy, 1998). However, the amount of residual protein often depends on the types of processing, and moreover the sensitization can occur even with small residues of carmine protein present in carmine-containing foods or cosmetic products (Acero et al., 1998; Taylor & Dormedy, 1998). Thus, one or more of those carmine-associated proteins would finally direct towards the carmine-specific IgEs to elicit IgE-driven allergic reactions, including asthma and anaphylaxis (Chung et al., 2001). In fact, there have been a number of cases of allergic reactions to carmine following the ingestion of carmine-containing alcoholic beverages, yogurts, popsicles as well as cosmetics, such as eyeshadows and lipsticks (Wuthrich et al., 1997; Beaudouin et al., 1995; Park, 1981; Kagi et al., 1994; Baldwin et al., 1997). Each reported case involved a series of skin prick test and specific IgE test to carmine whose results were all positive and especially the release of histamine following an exposure of patients' blood basophiles to carmine was confirmed through a leukocyte histamine release test (Belluco et al., 2013). According to Beaudouin and others (1995), the acceptable daily intake of carmine is up to 5.0 mg per kg of body weight, but the case caused by carmine-containing yogurt was triggered by 1.3 mg of carmine in the yogurt, which proves that there has been sensitization with a small amount of carmine and in the end such a small amount was enough to elicit allergic reactions, including life-threatening anaphylaxis. Chung and others (2001) demonstrated immunoblotting and immunoblotting-inhibition experiments, indicating that several different proteins originated from cochineal insect were present in commercial carmine. In addition, patients with allergic reactions against carmine as an additive in foods or cosmetic products, had IgEs targeting "protein impurities" in the extracts. Therefore, further studies are necessary to investigate whether pure carmine extracts without any protein would trigger IgE-mediated allergic reactions or not. Otherwise, it would be difficult to avoid any food containing carmine dye under current FDA labelling requirements for food (Chung et al., 2001).

Lee and others (1999) investigated cases of oropharyngeal manifestations caused by ingestion of caterpillars in children, based on 733 patients reported at Pittsburgh Poison Control Center

of the US, between 1994 and 1997. In the same year, Pitetti and others (1999) also described 26 cases in children following accidental or curiosity-driven intake of caterpillars and cocoons. Ingestion or oropharyngeal contact of caterpillars in children led to local and general adverse effects, including drooling, difficulty in swallowing, generalized urticaria and even shortness of breath (Lee et al., 1999; Pitetti et al., 1999). The mechanisms of local and general reactions were unknown, but it was suggested that a series of reactions resulted from combined effects of mechanical irritation by spines and followed hypersensitivity reaction to antigens present in the spines as well as envenomation from direct or indirect contact with aerosolized spines (Kawamoto & Kumada, 1984; Pinson & Morgan, 1991; Werno & Lamy, 1993; Lamy et al, 1983; Kawamoto et al., 1978). Moreover, the thaumetopoein protein has been isolated from pine processionary caterpillars, which elicited direct mast cell degranulation (Lamy et al, 1983; Kawamoto et al., 1978). Although reported cases suggested underlying allergic symptoms, Belluco and others (2013) criticized the lack of consideration in possibilities of toxic reactions in the two studies by Lee and others (1999) and Pitetti and other (1999). In order to distinguish between those possibilities, an allergological test is recommended, yet the test was not performed for both studies. On the other hand, caterpillars are the most commonly eaten insect group and an important protein source of the daily diet in central Africa (van Huis et al., 2013).

Caterpillars (Lepidoptera) are ingested entirely in their larva or nymphal stages (van Huis et al., 2013). The mopane caterpillar (*Imbrasia belina*) is the most popular caterpillar consumed with nutritional and economic importance in Africa (van Huis et al, 2013). The bamboo caterpillar (*Omphisa fuscidentalis*) is also being promoted by the Thai Department of Forestry of the Ministry of Agriculture and Cooperatives (Yhoung-Aree & Viwatpanich, 2005). Among more than 165,000 species of caterpillars, there are about 150 species that may induce certain irritant or toxic dermatitis (Lee et al., 1999; Pitetti et al., 1999). A few allergic manifestations have been described following ingestion of “edible” caterpillars in spite of a large amount of allergic manifestations caused by accidental ingestion of “in-edible” caterpillars (Okezie et al., 2010; Kung et al., 2011).

Silkworms have a long history of domestication dated back 5,000 years in China due to silk fabric production. Pupae of silkworm (*Bombyx mori*) also have been consumed as a delicacy in Asian countries, including China, Japan, Korea, Thailand and Vietnam (Belluco et al., 2013; van Huis et al., 2013). The silkworm pupae are processed, packed and labelled for sale and wholesale in the above mentioned countries (van Huis et al., 2013). They are now regarded as

a commercially viable product even for non-textile purposes, such as pharmaceutical and nutritional ends (BACSA, 2011).

In the Republic of Korea, where there is no specific culture of edible insects, silkworms have been the one and only insect-based snack placed for sale and wholesale, and recently produced as a medicine in the form of powder for diabetic patients based on several studies demonstrating its ability to reduce the blood glucose level (Ryu et al., 2012). However, allergic manifestations following ingestion of commercial silkworms in Korea have been hardly reported nor studied whereas in China, it has been estimated that about 1,000 patients each year experienced allergic reactions after the intake of silkworm (Ji et al., 2008). All edible silkworms for sale or wholesale in Korea have been approved as “safe food” by the Ministry of Food and Drug, but there have been no fundamental researches in relation with food safety of commercial edible silkworm pupae. According to personal inquiry at the Korean FDA, the background for approval lied only upon the following statement, “*edible silkworms have been safely consumed by Korean people over 30 years without officially reported cases of allergic manifestation following ingestion of the silkworm pupae*”. Besides, a request about “edible silkworms” and “allergic reactions” on the research engines produced a variety of non-official articles and comments about personal allergic experiences. The phenomena suggested that allergic manifestations including severe anaphylactic reactions to edible silkworms may be more frequent than it is perceived by very rare publications or scientific literature. Commercialized, can-preserved silkworms that are currently found in South Korea are 100 percent exported from China. Along with a decline of sericulture industry in the beginning of the 1980s (Choi, 2011), the market of edible silkworm has relied on exports, although Korean silkworms are still sold on traditional local markets or in medicinal herbal shops. The labelling of commercial edible silkworm pupae in Korea includes a statement indicating “*a particular warning of adverse effects after intake of silkworm pupae is susceptible to individuals with history of allergic diseases*”. In addition, the Korean Ministry of Food and Drug Safety has to ensure the safety of the nation with consumer-based safety management from farm to table. So, prior to promote edible insects as safe food, it should conduct larger scaled researches and studies on potential adverse effects or any anomalies following intake of insect food or feed for future consumers.

13 patients have been involved in severe anaphylactic reactions following intake of silkworm pupae in China, among which a French visitor ate oil-fried silkworm pupae for the first time in his life (Ji et al., 2008). One possible explanation implicates the cross-reactivity between certain allergens present in taxonomically dispersed insects (Belluco et al., 2013). Liu and others (2009)

identified arginine kinase from silkworms, which cross-reacts with arginine kinase of cockroach. Arginine kinase is a well-known pan-allergen with enzymatic properties and cross-reaction exists between the american cockroach (*Periplaneta Americana*), german cockroach (*Blattella germanica*), indian meal moth (*Plodia interpunctella*), silkworm (*Bombyx mori*) and mites (Liu et al., 2009; Verhoeckx et al., 2013). With respect to tropomyosin related cross-reaction, the same authors investigated that among 14 patients who had anaphylactic reactions after intake of silkworm pupae, less than 12% of them reacted with the invertebrate tropomyosin by immune-assaying technique (Liu et al., 2009).

Insect allergens related to consumption of insects as food or feed remain poorly studied and understood. Nonetheless, there have been still cases in connection with food allergy, mostly caused after intake of larvae of red palm weevils (*Rhynchophorus ferrugineus*), mealworm species, mopane worms (*Gonimbrasia belina*), silkworm (*Bombyx mori*) and superworms (*Zophobas morio*) (ANSE, 2015). In any case, we can always pursue safer choices for ourselves as well as reared animals with insect meal. For instance, avoiding phanerotoxic species, or their body parts containing antigenic venoms, or choosing certain developmental stages lacking stings (Belluco et al., 2013). Additional care is necessary for susceptible individuals, for instance, ingesting honeybee larvae should be avoided in pollen-allergic individuals since honeybee larvae often contain pollen (Chen et al., 1998).

4.4. Microbial hazards

Insects, like any other animals or plants on this planet are associated with a variety of micro-organisms throughout their life (van Huis et al., 2013). If insects have to be accepted as a daily food or feed ingredient with the agreement of local culture or legislation, insect-associated micro-organisms have to be taken into account since they may become causative agents of food-borne diseases. When insects will be commonly farmed in mass rearing industry, any disease caused by insects will be considered as a foodborne disease and create public health problems at both national and international level. According to the American Centers for Disease Control and Prevention (CDC), there are already more than 250 different foodborne diseases identified worldwide. Most of reported diseases are infections caused by various bacteria, viruses, parasites and fungi (Vega & Kaya, 2012).

4.4.1. Bacterial hazards

Consumption of edible insects can potentially transmit bacterial agents (van Huis et al., 2013). These bacterial agents are related to the intrinsic flora of insects in the gastrointestinal tract or anatomical compartments, or may have an extrinsic origin, such as microbes present on the external cuticles or exoskeleton of insects possibly obtained during the rearing, processing and storage conditions, including substrates of diet and handling by workers (ANSE, 2015). Such infections concerning insects are not cases of health hazards that may endanger animals and humans (van Huis et al., 2013). It is because insects and animals are phylogenetically very distant (ANSE, 2015), which means insects pathogens are taxonomically separated from vertebrate pathogens (van Huis et al., 2013). For example, within the genus Bacilli, the insect pathogenic *Bacillus thuringiensis* and the vertebrate pathogenic specie *B. anthracis* have non-overlapping life cycles (Jensen et al., 1977). On the other hand, Bacilli causing anthrax (*B. anthracis*) or food poisoning (*B. cereus*) can be transmitted by insects when they are contaminated from the environment, such as soil or during the food chain process (ANSE, 2015). However, there is hardly zoonotic infection between insects and vertebrates (ANSE, 2015).

Concerning bacteria spores, they may be present on the cuticle of insects and then be transmitted to animals and humans through handling or ingestion (van Huis et al., 2013). Therefore, all micro-organisms with the external origins absolutely need to be studied and controlled during the food or feed chain. Even during the post-purchase life of edible insects, it is necessary to educate consumers or clearly state on the product label about the importance of safe handling and storage.

Insect gut flora contains relatively few species of bacteria compared to those of mammals, but there is still a large number of population (varying 10⁸ to 10¹¹ per ml of intestinal content) (Berkvens et al., 2015). The gut flora of insects mainly consists of Gram-positive cocci and Gram-negative rod bacteria (Cazemier, 1999). Dillon & Charnley (2002) also isolated Gram-negative anaerobic Enterobacteriaceae (*liquefaciens*, *cloaca*, and *agglomerans*) with main bacterial groups; Gram-positive cocci and Gram-negative rod bacteria from the intestinal tract of cultivated locusts (*Schistocerca gregaria*). Putting up all publications by December 2014, the most common 5 species of cultivated, edible insects, which are house crickets (*Acheta domesticus*), locusts (*Locusta migratoria*), morio worm (*Zophobas morio*), waxmoth worm (*Galleria melonella*) and yellow mealworm (*Tenebrio molitor*), have been investigated concerning their microbiological flora in the quantitative and qualitative perspectives (ANSE, 2015; Belluco et al., 2013). The micro-biological flora from these fresh, cultivated 5 species

contained mostly faecal and total coliform of Gram-negative bacteria and Gram-positive bacteria, including *Micrococcus* spp., *Lactobacillus* spp., and *Staphylococcus* spp. (ANSE, 2015; Belluco et al., 2013). In another study conducted with cultivated meal worms (*Tenebrio molitor*) and house crickets (*Acheta domesticus*), Klunder and others (2012) isolated Enterobacteriaceae (104-106 cfu/g) and spore-producing bacteria (102 – 104 cfu/g), which do not belong to a pathogenic group in insects. A low value of less than < 10 cfu/g of Enterobacteriaceae was measured from raw silk worms (*Bombyx mori*) as well (Berkvens et al., 2015). However, either *Salmonella* or *Listeria monocytogenes* were never found in the 5 samples of edible insects (Giaccone, 2005).

Salmonella and *campylobacter* have been connected with poultry flocks as an important pathogenic species (Belluco et al., 2013). *Salmonella* is persistently carried by flies living near *Salmonella* contaminated flocks as well as by beetles, but in a lesser extent (Belluco et al., 2013). Extensive experimental evidences support arthropod-mediated transmission for both *salmonella* and *campylobacter* (Wales et al., 2010). Thanks to Templeton and others (2006), darkling beetles (*Alphitobius diaperinus*) were identified as both reservoir and vehicle of *campylobacter* originated from faeces of farmed broilers.

The poultry-originated *campylobacter* infects both larvae and adult of darkling beetle while the infected darkling beetles transmits *campylobacter* to poultry as well (Belluco et al., 2013). Zoonotic infection of *campylobacter* between *A. diaperinus* and poultry has become a significant foodborne disease in Europe (EFSA, 2012). Furthermore, *campylobacter* is one of the most important foodborne pathogens in Australia and New Zealand where the poultry is responsible for 40% of foodborne illnesses in humans (Belluco et al, 2013). Therefore, specific attention needs to be paid to *A. diaperinus* as reservoir and vehicle of *campylobacter* although they have limited ability to harbour *campylobacter* (Hazeleger et al, 2008; Templeton et al, 2006). Microbiological analysis of both larvae and adult of *A. diaperinus* was demonstrated by Agabou and alloui (2010). *A. diaperinus* carried multiple pathogens to poultry, as possessing natural flora of the gastro-intestinal tract and other pathogenic species on the external surfaces. The natural inhabitants in the GI tract of larvae were composed of coliforms of Gram-negative bacteria and some streptococci, and the exterior part of larvae was contaminated with *micrococcus* spp. and *staphylococcus* spp. (Belluco et al., 2013).

Salmonella arizonae were found on the exterior cuticle of 5 % of adult *A. diaperinus* whilst no thermophilic *campylobacter* species were identified (Agabou & alloui, 2010; Giannella, 1996). *A. arizona* is one of 6 sub-species of *Salmonella enterica* to which most of human pathogenic

serovars belong (Agabou & alloui, 2010; Giannella, 1996). Through a study conducted in 1986, *A. diaperinus* has become known as an insect species highly contaminated with bacteria on average (Belluco et al., 2013; Goodwin & Waltman, 1996). Larvae of *A. diaperinus* (Tenebrionide family) are being actively reared as feed for reptile, fish and avian pets in the Netherlands (van Huis et al., 2013). Indeed, *A. diaperinus* are currently being sold as freeze-dried with two other species, the yellow mealworm (*Tenebrio molitor*) and the migratory locust (*Locusta migratoria*) for human consumption on Dutch markets (van Huis et al., 2013). Therefore, specific microbiological screen for *A. diaperinus* should be strictly performed at a regular basis since they are consumed by both animals and humans.

Concerning foodborne diseases, housefly (*Musca domestica*) has a significant role as mechanical vector of campylobacter (Nelson & Herris, 2007). However, another feeding experiment on houseflies with *Escherichia coli* 0157:H7 proved that houseflies may not only harbour *E. coli* 0157:H7, but also disseminate *E. coli* 0157:H7 (Kobayashi et al., 1999). This is an enterohemorrhagic serotype of *E. coli*, responsible for haemorrhagic colitis and haemolytic renal failure via consumption of contaminated water and food (Karch et al., 2005). *E. coli* 0157:H7 is well known for high virulence and low infectious dosage (Greig et al., 2010). Muhammad and Ludek (2004) concluded that housefly plays an important role in the dissemination and the transmission of *E. coli* 0157:H7 among individual cattle within a flock, and then to the surrounding farms as well as urban environment (Muhammad, & Ludek, 2004).

FAO's Animal Feed Resources Information System recently suggested that edible insects including common housefly maggots can be a great feed source to aquaculture species (van Huis et al, 2013). Besides their nutritive value, maggots of fly species have a vital role in bio-degradation of organic wastes and animal manure. Therefore, the European Commission funded project is being on the way to figure out how larvae of housefly can be used to recycle organic waste into fertiliser and even into biofuel (van Huis et al, 2013). At the same time, it largely implicates to develop more quantitative risk assessment as well as hygienic management program when such bio-degrading insects, including fly maggots, larvae of beetles, termites and ants become common food and feed ingredients since they may pick up pathogens and contaminants and then transmit them to their consumers.

4.4.2. Fungal hazards

The most frequently isolated species are *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Phycomycetes*, among which strains of *Aspergillus*, *Fusarium* and *Penicillium* are associated with mycotoxin production (van Huis et al, 2013). The fungal agents are first contaminated by leaves and soil, and then re-contamination occurs during drying and storage process in poor conditions (Simpanya et al., 2000). Mpuchane and others (1996) conducted a study investigating the types and level of moulds and aflatoxins isolated from a sample of edible caterpillars (*Imbrasia belina*) in Botswana (Mpuchane et al., 1996). The caterpillars in this experiment were degutted and boiled for a maximum of 30 minutes, then spread out on a sheet for 1 to 3 days of sun-dry (Mpuchane et al., 1996). Nonetheless, aflatoxin-producing *Aspergillus* species, such as *A. flavus* and *ochraceus* were highly prevalent in the ready-to-eat phane (caterpillars of *Imbrasia belina*), followed by *Rhizopus*, *Absidia* and *Mucor* species of the *Phycomycetes* (Mpuchane et al., 1996). Some of samples collected from different localities contained aflatoxins whose concentration varied from 20 to 50µg of aflatoxins per kg of sample, whereas 20µg of aflatoxins per kg of sample is considered as the maximum tolerable amount in most countries (Mpuchane et al., 1996).

Consumption of fungal contaminated food with a frequency over a long period is likely to provoke health hazards with various degrees in severity (ANSE, 2015). Moreover, some mycotoxins are known to cause acute intoxication following intake of heavily contaminated foods with fungal species producing mycotoxins (Mpuchane et al., 1996). Mycotoxins, which are toxic secondary metabolites produced by certain species of fungi, includes aflatoxins (B1, B2, G1, G2 and M1), fumonisins (B1, B2 and B3) ochratoxin A, patulin, trichothecenes (mainly nivalenol, deoxynivalenol, T-2 and HT-2 toxin) and zearalenone (Bennett & Klich, 2003). They can cause adverse effects, including mutagenicity, carcinogenicity and organ toxicity depending on species of fungi and types of mycotoxins (Peer & Linsell, 1973; Gelderblom et al., 1988; Jay, 1991). Mpuchane and others (1996) therefore high-lightened on the importance of proper management during processing, handling, drying and storage to reduce fungal infestation.

Based on methods recommended by FAO, cooked caterpillars of the mopane moth (*Imbrasia belina*) were dried properly at a safe moisture level and during storage a special attention was paid to reduce the moisture level by frequent aeration in order to avoid cross-contamination of different products (FAO, 1979; Gourama & Bullerman, 1995; Mpuchane et al., 1996). Most of existing documents related to fungal contamination by insect consumption have been collected

in the wild or harvested with traditional methods. Therefore, potential fungal hazards can be mitigated with strict hygienic measures during the food processing and storage. Moreover, industrialization of rearing system will allow even greater control over food safety, particularly on fungal contaminations that are greatly influenced by nutrient medium and environments of habitation (ANSE, 2015).

4.4.3. Parasitic hazards

There have been only sporadic cases concerning parasitic hazards caused by intake of infected insects. There is still more knowledge about vector-borne, parasitic diseases where insects play a role as vector in the transmission of diseases. According to World Health Organization (WHO), the majority of vectors in parasitic diseases are insects, including mosquitoes, flies, sand-flies, fleas and kissing bugs. Moreover, some insects are considered as parasitic agents, such as fleas, lice, parasitic bugs, flies, wasps and some species of ants. Now, we are faced with another potential risk of parasitic infection when insects are ingested as food or feed sources. Along with the establishment of industrial scaled rearing system in the near future, insects are about to be mass-produced all over the world. Therefore, insect-borne parasitic infections or diseases may become an important part of food-borne diseases at the level of public health concern.

Parasitic hazard to metacercariae and cercariae is often manifested by insect vectors, such as aquatic insects or insects living near water, for which host reservoirs can be certain local species of birds or fishes. Chai and others (2009) reviewed about foodborne intestinal flukes in Southeast Asia, where a long tradition of insect consumption has been continued until now. 6 out of 64 species of food-borne intestinal flukes were identified from local insect samples (Chai & others, 2009). In particular, metacercariae and cercariae of *Phaneropsolus bonnie* (Lecithodendriid) were isolated from naiad (an immature nymphal stage) and adult of dragon and damselflies in northeast Thailand, where those insects are commonly consumed (Chai et al., 2009). 15 more cases of the same fluke (*Phaneropsolus bonnie*) were confirmed during human autopsy in the same area of northeast Thailand (Belluco et al., 2013). It turned out that dragon and damselflies also harbour another fluke species of Lecithodendriid flukes (*Prosthodendrium molenkampii*) (Belluco et al., 2013), and its high prevalence was described in different areas of northeast Thailand. In 1991, a new specie, *Phaneropsolus spinicirrus* provoked one novel case, as metacercariae of *Phaneropsolus spinicirrus* was identified from naiad and adult of dragon and damselflies in the same region (Kaewkes et al., 1991). Another

parasitic infection due to a new family of fluke, Plagiorchid was first reported from human case during the treatment of 4 opisthorchiasis patients with praziquantel (Radomyos et al., 1989). It turned out to be a species called *Plagiorchis javensis* (Family Plagiorchid) which might be originated from insect larvae as a second intermediate host, for which local birds and fishes are the reservoirs (Belluco et al., 2013).

Parasitic infection to nematode, *Gongylonema pulchrum* was the only genus reported through 50 human cases, for which Coleoptera and Blattodea play a role in intermediate host (ANSE, 2015). They are ubiquitous, as infecting wild and domestic mammals, including ruminants, rabbits, dogs and cats (Belluco et al., 2013). Intermediate hosts are often coprophagous insects, such as dung beetles and cockroaches, which ingest eggs shed in the faeces of infested animals (Belluco et al., 2013). As the larvae 1 (L1) become released into the insect vectors, they develop into L3 which will be ingested with insects by a variety of mammals. This zoonotic infection may be acquired by ingestion of raw or improperly cooked insect vectors (ANSE, 2015). The clinical features in humans are mainly subcutaneous larva migrans in the oral cavity (Wilson et al., 2001).

Battyany and others (2001) reported an infrequent case of Hydatid cysts developed in the human subcutaneous tissues, following a wasp sting. The Hydatid cysts turned out to be *Echinococcus hydatidosus*, the larva form of *Echinococcus granulosus*, whose common path is known as the ingestion of parasite eggs present in contaminated water or food, or through direct contact with an animal host, such as canids (McManus et al., 2003). Human Echinococcosis is a zoonotic disease through the handling of faecal matters of infected canids or ingestion of infected animals (WHO, 2015). Thus, such transmission of the eggs by insect-sting is considered as rare, yet it should not be overlooked, especially in endemic areas (Battyany et al., 2001).

Myiasis is another parasitic infestation with larvae of Diptera on the live body of humans or vertebrate animals (Sehgal et al., 2002). As flies shed eggs on open-wound, urine- or faeces-soaked fur, even unbroken skin, their larvae develop by feeding on live tissues of vertebrate hosts (David & William, 2006). In case of intestinal myiasis, ingested fly eggs reach the GI tract and pass within the faeces as larvae (ANSE, 2015). Therefore, intestinal myiasis is diagnosed by presence of fly larvae within stools of host while it is frequently asymptomatic and transient (Belluco et al., 2013). Otherwise, the three main fly families, which are Calliphoridae (Blowfly), Oestridae (Botfly), Sarcophagidae (Fleshfly), have an economically significant implication in livestock farms. Besides the main fly families, there are *Eristalis tenax*

(Drone fly), *Hermetia illucens* (Black soldier fly), *Megaselia scalaris* (Scuttle fly) and *Phormia regina* (Black blowfly) (Sehgal et al., 2002).

Lupi (2006) pointed out myiasis as a risk factor of prion diseases in humans through the laboratory experiments. It was proved that certain ecto-parasitic insects, including fly larvae and pupae, can harbour prion rods (Lupi, 2006). The skin and mucous membranes are the potential targets of prion infections since keratinocytes and lymphocytes are susceptible to the abnormal infective isoform of prion proteins (Lupi, 2006).

The foodborne and waterborne protozoan agents have to be taken into account as health hazards in connection with certain insects, such as cockroaches and flies which can harbour such parasitic protozoa and potentially transmit them to humans and other animals (ANSE, 2015; Belluco et al., 2013). Among protozoa being spread in food or water, *Cryptosporidium*, *Cyclospora*, *Giardia*, and *Toxoplasma* are considered as a great risk to food production worldwide (Dawson, 2005). Other parasitic protozoans are not considered as such a risk for humans and animals even though they may be present and spread through food and water (David, 2005).

Concerning foodborne or waterborne pathogens, houseflies and synanthropic cockroaches are the most frequently screened insects (Yiu, 2006). Cockroaches are known to carry pathogenic protozoa, such as *Entamoeba histolytica* and *Giardia lamblia* (Belluco et al., 2013). Pai and others (2003) also recovered viable cysts of *Entamoeba histolytica* from the external surfaces and guts of cockroaches (*Periplaneta Americana* and *Blattella germanica*). Those cockroaches harbour *Toxoplasma* spp. and *Sarcocystis* spp. (Belluco et al., 2013; Graczyk et al., 2005).

A variety of pathogenic protozoa have also been found in flies, among which *Cryptosporidium*, *Giardia*, *Isospora*, *Sarcocystis* and *Toxoplasma gondii* (Belluco et al., 2013). Despite humans are not the primary hosts of *Toxoplasma gondii*, Toxoplasmosis in humans can cause a specific risk via congenital transmission, especially when pregnant female are infected. The infection is manifested by a systemic disease to foetus with various degrees of severity (Lopez et al., 2007).

Another protozoan pathogen to be needed a particular attention is *Cryptosporidium parvum* causing diarrheal cryptosporidiosis (CDC, 2015). It is a leading cause for the most important waterborne disease in developing countries (CDC, 2015). If the infection itself is very self-limiting in immunocompetent people, yet it is not in immune-compromised individuals or patients undergoing any kind of immune-suppressive therapy (Graczyk et al., 2005). It is thus very lethal as causing dehydration and even death in a serious case in relation with the

immunological condition of individuals (Graczyk et al., 2005). Those main parasitic protozoa do not multiply in foods, but can survive in cool and damp environment. They are sensitive to standard pasteurisation techniques (Dawson, 2005). Consumption of undercooked or raw cockroaches and flies should be avoided in compliance with Hazard Analysis Critical Control Point (HACCP) plans along the food and feed chain of edible insects.

Chagas disease or American trypanosomiasis has been considered as an important parasitic disease showing how insects can perform a biological vector (Belluco et al., 2013). The insect-vector is called triatomine bug or kissing bug, mainly found in endemic areas of Latin America (Argentina, Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guyana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Venezuela and Uruguay) (WHO, 2015a). According to the World Health Organization (WHO), about 6 to 7 million people are estimated to be infected worldwide, but at first it was entirely confined to Latin America. However, it has spread to other countries, including the US, Canada, the EU and some Western Pacific countries in the past decade (Schmidt, 2010). The causative protozoa of Chagas disease, *Trypanosoma cruzi* are transmitted by blood-sucking or contact with faeces/urine of infected kissing bugs (ANSE, 2015).

Although it has been 100 years since the first discovery of the disease by Brazilian Physician Carlos R. J. Chagas, very little has been invested since the main habitat of the insect vector is cracks of poorly constructed housings in developing countries (Schmidt, 2010). In between, Chagas disease has become a public health concern worldwide as recent cases were reported in relation with the ingestion of fruits or vegetables contaminated with *T. cruzi* via contact with infected kissing bugs or their faeces/urine (Pereira et al., 2010). Birds and other wild animals in endemic areas appear to be host reservoirs in the sylvatic cycle (Schmidt, 2010). Recently, University of Texas at El Paso (UTEP) researchers conducted a study showing that the US are no longer safe from Chagas disease even with their sturdier housings (Buhaya et al., 2015). According to their findings, infected kissing bugs have already been prevalent throughout the Southern US and 24 mammal species were identified as reservoirs of *T. cruzi* (Buhaya et al., 2015). Another laboratory experiment demonstrated that bed bugs, which are ubiquitous all over the world, can actually transmit parasitic protozoa to laboratory mice and non-infected bed bugs also picked up *T. cruzi* from infected mice (Buhaya et al., 2015).

4.4.4. Viral hazards

According to ANSE report (2015), there is no documentation on the viral risk caused by handling or consuming insects.

4.5. Microbiological aspects of processing and storage

A variety of health hazards associated with the ingestion of insects as food or feed are investigated, based on reported cases all over the world. Chemical, physical and allergenic hazards following intake of insects are not likely mitigated with processing techniques, frequently cooking or acidic fermentation while parasitic hazards can be reduced with heating or boiling processes, but more importantly, it is necessary to consider an introduction of efficient deworming plans encompassing different conditions as species, developmental stages and geographical factors at the rearing level. Among which, bacterial hazards are relatively well controlled under safety management during the total steps of the food or feed chain. It is known to inactivate or reduce the microbial content by cooking processes, such as boiling and roasting, or pasteurization (Giaccone, 2005).

Klunder and others (2012) emphasized the significance of processing and storage conditions in connection with a much longer shelf life of alimentary products in these days. They have conducted a laboratory experiment with the comparison of the microbiological status between whole fresh, processed and stored edible insects. The microbiological contents of two species of edible insects; the yellow mealworm (*Tenebrio molitor*) and the house cricket (*Acheta domesticus*) were analysed as fresh, boiled, roasted, fresh and stored at a refrigerated and ambient temperature, and finally boiled and stored at a refrigerated and ambient temperature as well. Enterobacteriaceae and spore-forming bacteria were still identified in fresh samples, yet they did not belong to pathogenic species. Thermal treatment as fresh samples being in boiling water for 5 minutes eliminated Enterobacteriaceae, but not spore-forming bacteria. Those soil-borne spore-forming bacteria are quite resistant to diverse environmental conditions and generally have the ability to germinate and cause food spoilage at an optimal environment, for instance around 30 degree Celsius with enough humidity. Hence, storage at refrigerated temperature is suggested since this will prevent food spoilage by spore-forming bacteria (Belluco et al., 2013). Roasting process did not eliminate all Enterobacteriaceae, it is thus recommended to boil insects for a few minutes prior to roasting (Klunder et al., 2012).

Lactic fermentation as an alternative preservation technique showed relatively efficient results for controlling both Enterobacteriaceae and spore-forming bacteria (Klunder et al., 2012). According to the same authors, the technique is composed of flour and water mixtures with 10 to 20% of powdered, roasted mealworm larva, followed by a successful acidification and inactivation of Enterobacteriaceae. The lactic fermentation technique also stabilized the acceptable level of spore-forming bacteria by preventing them from germination. As another non-heating technique, the microbiological screening of dried and ground insects was operated (Klunder et al., 2012). And drying and grinding process resulted in a moderate amount of microbes, but the microbiological status remained constant even at room temperature.

5. Legal requirements

FAO's paper (2013) on edible insects has a great implication on regulatory barriers that actually need to be re-assessed and adapted for the development of edible insect-market in the world. In developing countries, especially where edible insects contribute to satisfy the local protein needs, the use of insects for food and feed has quite a high degree of tolerance, since insects have never been correctly documented in food policies, while nature conservation is an issue of high importance (Halloran et al., 2015). In developed countries, where edible insects were not recognized as food and feed resources, insects have never been incorporated into policies or have been largely omitted from the entire regulatory frameworks (Halloran et al., 2015). Therefore, complex regulations hinder the establishment of edible insect-industry at both national and international level.

In May 2014, the Food and Agriculture Organization (FAO) of the United Nations and Wageningen University of the Netherlands hosted the "Insects to Feed the World Conference" in Ede (The Netherlands) for mainly discussing about issues to be faced in relation with edible insects for animal and human consumption (FAO/WUR, 2014). A majority of participants pointed out the major issue to be insect inclusive food and feed legislations (Halloran et al., 2015).

5.1 Current issues related to edible insects as feed: European perspectives

In general, there has been a lack of insect inclusive legislations or clear norms guiding their production and trade as food and feed in the European regulatory contexts (Halloran, 2014). The reason was mainly as insects were not recognized as food and feed sources and consequently, there was no demand for edible insects of that purpose. Therefore, the new rising interest in edible insects may increase demand for them and then, the re-frameworks on related regulations with a risk assessment will be implemented (ANSE, 2015).

For the last decades, regulatory frameworks which govern the use of certain raw materials or ingredients have become a very crucial factor influencing the consumption patterns (ANSE, 2015). Due to globalization, food chains are longer and more complicated than ever (van Huis et al., 2013). Correspondingly, consumers' concerns and interests towards food quality and safety have grown dramatically, yet no relevant food policies are present for edible insects (van Huis et al., 2013).

Concerning the insect-based feed ingredients in the EU Regulations, there are no references stating “insects” on the list for restriction of processed animal proteins (PAPs) directed to farmed fish and non-ruminant livestock (ANSE, 2015). Edible insects are now being considered as an alternative and sustainable protein source for livestock, but until recent days, only fishmeal, fish oils, soybean meal and other grains have been included in the diet of farmed animals (Belluco et al., 2013). MIT Technology Review pointed out that an insect-based feed will be a near perfect replacement of fishmeal and soybean meal which are typically used today but are more expensive and less ecological than them. In spite of such enthusiasm towards the positive future of edible insects in animal feed industry, the lack of legislative standards and regulatory instruments hinder this industry to grow (Belluco et al., 2013).

Moreover, some edible insect species can be reared on organic wastes and animal manures which are parts of their natural diet. Bio-degradable edible insects are expected to be a great opportunity to reduce the volume of organic wastes worldwide. However, the Transmissible Spongiform Encephalopathies (TSE) Regulation blocks the approval of such rearing system (Regulation (EC) No 999/2001) due to unknown risks in transmission of pathogens and contaminants. Therefore, it is an inevitable process to review the existing regulations through a re-assessment of potential health hazards while complying the correct procedures in terms of food safety and animal welfare.

In addition, no specific guidelines have been given for the setting up of insect farms, prior to the establishment of a mass rearing system. There are thus no national animal protection measures specifically for insects in captivity. Furthermore, insects are covered by “captive wildlife regulations” (van Huis et al., 2013), since they are not classified as domestic species. Apart from establishing a mass rearing facility for insects, “the grants of a farming competency certificate and a prefectural authorization of opening” have also to be developed (ANSE, 2015).

First of all, insects are not listed in the catalogue of feed material, Commission Regulation (EC) No.68/2013. Insects thus cannot be placed on the European markets for food and feed. However, “*terrestrial invertebrates*” are mentioned in entry 9.16.1 as “*whole of parts of terrestrial invertebrates in all their life cycles, other than species pathogenic to humans and animals; with or without treatment such as fresh, frozen, dried*” (van Huis et al., 2013). Therefore, insects, when they are not pathogenic to humans, can be classified as Category 3 in Article 10.1 of Regulation (EC) No 1069/2009 (2009) since insects are parts of “*terrestrial invertebrates*”. Nevertheless, there is still an ambiguity as the terms “insect meal” were never mentioned nor pointed out in the regulations. Moreover, insect meal is regarded as processed animal proteins

(PAPs) whilst they are included in Category 3, which are suitable to be given to livestock, by Article 10.1 of Regulation (EC) No 1069/2009 (2009). On the contrary, Regulation (EC) No 999/2001 (2001) prohibits the use of PAPs in livestock feed, which means that they cannot be given to food-producing animals, but may be given to pets. Strictly speaking, as insects are not “mammals” nor “vertebrates”, they should not be regarded as PAPs, which allows to give them to food-producing animals. Regulation (EC) No 999/2001 (2001), also referred as “BSE regulation”, bans the use of proteins originated from mammals as feed for livestock in the EU. Bovine Spongiform encephalopathy (BSE), which is better known by the public as “mad cow disease”, was out-broken between the 1980s and 1990s as bovines were fed by extracted proteins coming from the same species (Belluco et al., 2013). Hundreds of people then became infected by consuming infected bovines and caught a human form of BSE, variant Creutzfeldt-Jakob (van Huis et al., 2013). This was the reason why EU banned all types of animal proteins to feed livestock. Antoine Hubert, a French scientist and the co-founder of Ynsect, a company that aims to construct an industrial scaled insect-rearing system, explained that the risk of transmission of prions or other pathogens between insects and mammals is much smaller than between mammals themselves (ANSE, 2015). There are no known prions that may develop diseases in insects, based on scientific literature (ANSE, 2015). Hubert also emphasized that insect-specific bacteria or viruses are not likely to harm humans (ANSE, 2015). One should also be aware that possible problems liable to be caused by other pathogens that insects pick up through their diets can be mitigated by a careful selection of the feed sources given to insects (Kupferschmidt, 2015).

Until 2012, feed companies operating in the EU have kept insect meal only to pet food. However, the potential of insect as feed is too remarkable to be neglected only because of regulatory uncertainty. The IPIFF¹ association asked for the revision of the EU’s insect-feed regulation so that insect meal could be used as feed in livestock, with a compromise stating that insects will be fed with 100% vegetable substrates during production (IPIFF, 2014). The high level representatives and sketch-holders of the EU institutions agreed on a modification of Regulation (EC) No 999/2001 (2001), which bans the use of PAPs for the food-producing animals. Therefore, edible insects, which are fed with 100% vegetable substrates, have become allowed for feeding aquaculture species since June 2013 by Regulation (EC) No 56/2013 (2013). However, one problem emerged as insects directed to fish feed have to be slaughtered in a certified house with the presence of a welfare officer. Therefore, Regulation (EC) No 56/2013

(2013) gives an impossible criteria to be respected since insect-livestock are not likely killed in a slaughter house, but rather directly slaughtered in the rearing farms.

5.2 The Novel Food Regulation of the EU

It is significant to note that more than 2 billion people in the world already consume insects as daily diets (Day, 2015). Currently, “2,086 species of insects are consumed by 3,071 ethnic groups in 130 countries of the world” (Ramos-Elorduy, 2009; Rumpold & Schlüter, 2013), except mainly for European and North American countries. In such developed countries, the concept of “novel food” exists, which refers to food products that do not have a history of human consumption in the region or country in question (van Huis et al., 2013). This term includes edible insects and as well as products of biotechnology, including genetically modified foods (Belluco et al., 2013). It is easily understood that genetically modified foods are regarded as “novel food” worldwide since nobody can confirm the safety of those foods in case of human consumption. However, edible insects have been consumed by over 2 billion people around the world in such a long history (ANSE, 2015). At the same time, a long history of human consumption also proves that the intentionally reared insects for food or feed will not cause an important risk (Banjo et al., 2006). In the EU, novel foods and novel food ingredients are considered as that have never been used for human consumption to a significant degree before May 1997 within the EU, according to Regulation (EC) No 258/97 (1997), Article 3(1) of the European Parliament and of the Council. Hence, all insects are considered as novel food except for a few species of insects that have been reared in the EU before May 1997. On the other hand, certain species of insects, which are considered as novel food in the EU, cannot be a novel food if there is a human history of safe use in other countries. On this matter, the European Food Safety Agency (EFSA) will conduct a risk assessment as pre-market safety evaluation.

As novel foods or novel food ingredients, insects therefore require “a pre-market safety evaluation” and as well as “elaborating normative frameworks” of the already existing and above related food laws in order edible insects to be placed on the market of the EU (van Huis et al., 2013). The recent issues relative to edible insects raise more questions about the impreciseness in the insect-inclusive laws of the EU. Edible insects for human consumption come under Regulation (EC) No 258/97 (1997) and should be subject to the clarification of certain ambiguities in the regulation, such as the words “it covers only the parts of animals, not as whole insects”, which means that “a whole insect” is not regarded as a novel food (van Huis

et al., 2013). A meeting for a revision of the above regulation is scheduled in 2016 and these ambiguities will be discussed (Halloran, 2014). Therefore and until the European Commission reaches an agreement on the harmonization of novel food regulations, edible insects for human consumption cannot be placed for sale on the market across all EU member countries.

Food businesses in the EU selling insects for human consumption now need to prepare themselves for the amendments of novel food laws to come. EU manufacturers have to provide relevant information to the FAS (Food Standards Agency) that could convincingly demonstrate how such products have been already consumed at a significant degree in the EU history before the 15 of May 1997 (ANSE, 2015). Otherwise, they will be obliged to supply complementary information so as to demonstrate the safety of their products if they want to keep on selling them. Meanwhile, some manufacturers counterplot so as to take an advantage from the ambiguity of the regulation relative to “novel food” in order to exempt themselves from the procedures, prior to their marketing. For instance, the FASFC (Federal Agency for the Safety of the Food chain) of Belgium has authorized the marketing of ten insect species and their derivatives for human consumption (ANSE, 2015). From Dec 2013, Belgian’s insect-producing companies have benefited from this authorization under the guide of compliance with the general food legislation rules in force while waiting for the marketing authorization in the EU market as a whole (ANSE, 2015).

5.3 Codex Alimentarius

International standards for food and feed can be a useful reference to set up regulatory frameworks at national level. In this case, Codex Alimentarius particularly facilitates the trade of food and feed with compliance to trade rules (van Huis et al., 2013). The Codex Alimentarius Commission was established in 1963 by FAO (Food and Agriculture Organization) and WHO (World Health Organization) on the purpose of “*protecting the health of consumers and ensuring fair trade practices in the food trade*” (van Huis et al., 2013). Since 1963, the Codex Alimentarius Commission has developed harmonized international food standards, guidelines, and standards of practice, assisted by independent risk assessment bodies with the best available scientific competence in order to achieve the safety and quality of food and feed and as well as fair practice in food trade (van Huis et al., 2013). Concerning the subject of insects as food and feed, insects have been regarded only as “*impurities*” by Codex Standard 152-1985, stipulating that wheat flour shall be free of abnormal flavors, odors and living insects and as well as any

impurities of animal origin, including dead insects that may represent a health hazard to human health (van Huis et al., 2013). The only example for the incorporation of edible insects into international standards comes from Laos Popular Democratic Republic (PDR), proposing to develop a regional food standard for edible crickets and their by-products. This project was assisted by FAO UN, in collaboration with the Laos Ministry of Health (Halloran et al., 2015). After Laos PDR, several countries, such as Cambodia, Thailand and Malaysia volunteered to support that proposal (Belluco et al., 2014). Regarding the extensive cultural background of edible insect-consumption in Asia, Laos PDR proposition remains an important opportunity to include insects in the Codex Alimentarius (Halloran, 2014). CAC (Codex Alimentarius Commission) stressed on the point that adopting the Codex food standard and adapting it to regional standards may increase the level of food safety and the quality of edible insects in Asia (CAC, 2010).

5.4 European key administrative bodies

The European Food Safety Authority (EFSA) is an independent authority, established in 2002 by “*the EC General Food Regulation 178/2002 which lays down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety*” (Halloran, 2014). The EFSA task is to administer risk assessments regarding food and feed safety.

The European Commission Director General for Health and Consumers (DG Sanco) monitors “the implementation of EU Regulations at national, regional and local governmental levels” to ensure that traders, manufacturers and food producers obey the regulations (Halloran, 2014).

The Scientific Committee on Food is responsible for “*developing recommendations concerning food and feed safety*” (Halloran, 2014). Assessment of the quality and safety targets on “*environmental contaminants such as heavy metals, dioxins, PCBs and PAHs; pathogens and spoilage agents; chemical residues such as pesticides and veterinary drugs; allergens*” (ANSE, 2015). It also includes microorganisms in Hazard Analysis Critical Control Point (HACCP) plans along the different steps of the food and feed chain. The HACCO as a science-based system that identifies health hazards and measures for food safety (ANSE, 2015).

6. Conclusion

Edible insects are being recognized as a sustainable protein source to solve the world's food and feed security. Insect were never regarded as food or feed in developed countries while insect consumption is being practised in other parts of the world. Since FAO/WU's paper (2013), a global attention has been paid on their nutritional and ecological values.

A high level of high quality proteins and well-balanced fat composition make edible insects suitable for the diet of aquaculture and poultry species. Many trail studies proved that up to 25% of fishmeal or soybean meal can be replaced by insect meal in Aquaculture. In poultry, 25% of maggot meal in the diet yielded better live weights, feed intake and daily gain in comparison with the same amount of fishmeal.

Besides the high nutritional value of edible insects, insect production has a positive impact on the environmental sustainability. The production of insect meal costs almost a half of that of the fishmeal production in the level of EU and GHG production. The LU in insect meal has almost none while soybean meal production is remarkably land-intensive. There is no drinking water supply for rearing insects. Some insects species can be reared on organic side streams to bio-transform the organic wastes into bio-fertilizers and a high quality protein.

To counteract the chemical hazards following insect consumption, a controlled feed program should be considered with a careful selection of their nutrient sources and food contacting materials during the insect food and feed chain. Concerning the allergen hazards, appropriate indications of allergic risks in food labelling should be required. Unlike other hazards (allergen, chemical and physical), the microbial hazards can be mitigated by simple hygienic measures (cooking and/or freezing as appropriate). Klunder and others (2012) also demonstrated a laboratory experiment showing that storage at a refrigerated temperature following a thermal treatment in a boiling water for 5 minutes eliminated Enterobacteriaceae and kept the acceptable level of spore-forming bacteria without a food spoilage.

Concerning the industrial feasibility of edible insects as food and feed ingredients, challenges remain in the re-frameworks of regulations relative to edible insects as well as a safety assessment as food and feed.

7. Summary

Edible insects are becoming a key to solve the world's food and feed security responding to an increasingly growing world's population. Despite their nutritional value that is comparable to that of conventional meats and fish, insects have never been recognized as food or feed in developed countries. In fact, insect consumption has been a worldwide food habit, dated back to pre-history and is still practiced in many countries, even where food security is not a major concern. Moreover, insects are parts of the natural diet in conventional livestock, especially aquaculture and poultry species. To start with FAO/WU's paper (2013) on edible insects as a new food and feed ingredient, many researchers and associations have high-lightened in their publications that insects can be reared sustainably and more ecologically compared to other livestock.

Based on many documented studies with the 6 most promising edible insect species, insects generally contain a high level of high quality proteins with the presence of all the essential amino acids that are well-balanced to meet animal and human needs. Their fatty acid composition is comparable to that of fish, which are known to be healthy since the ratio between the 3 fat categories (SFA, MUFA and PUFA) remains within the suggested range. Compared to other food sources of animal origin, they have an important fibre content in chitin and chitosan.

The ecological footprint of edible insects can be evaluated with the following criteria, feed conversion rate and life cycle assessment. They have a high feed to meat conversion rate compared to other conventional meats. The production of insect meal and fishmeal results in high EU (Energy Use), which affects the GWP (Global Warming Potential). However, the EU and GWP of fishmeal indicated an almost double value as those for insect meal production. The LU in larval meal and fish meal recorded almost none while soybean meal production is remarkably land-intensive. There is no additional water use for the production of insect meal.

To counteract the chemical hazards following insect consumption, a controlled feed program should be considered with a careful selection of their nutrient sources and food contacting materials during the insect food and feed chain. Concerning the allergen hazards, presence of some allergens, which are common to different kinds of food, leads to cross-reactivity phenomena, for instance, between insects and crustaceans. Insects also can harbour a variety of food pathogens, including bacteria, parasites and fungi. In the light of animal husbandry practices, these risks can be reduced, since parasites and fungi are strictly connected to environmental features.

There are technical, economic and legislative challenges for insect meal producers, among which regulatory barriers need to be imminently re-assessed and adapted for the development of edible insect-market at both national and international level. From the European perspectives, there has been a lack of insect inclusive legislations or clear norms guiding their production and

trade as food and feed. Thus, further risk assessment of edible insects as feed ingredient is required to revise the current regulations and develop the regulatory re-frameworks.

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10. Appendices

Insect species described in the article and relative hazards (Belluco et al., 2013).

Order	Family	Genus	Species	Common name	Hazard category	Potential hazard	Reference
Coleoptera	Tenebrionidae	<i>Tenebrio</i>	<i>Tenebrio molitor</i>	Mealworm	Microbial	High bacterial count	Giaccone and others (2005)
		<i>Zophobas</i>	<i>Zophobas morio</i>	Superworm, zophobas	Microbial	High bacterial count	Giaccone and others (2005)
		<i>Alphitobius</i>	<i>Alphitobius diaperinus</i>	Lesser mealworm, Buffalo worm	Microbial	High bacterial count	Templeton (2006); Goodwin (1996); Agabou (2010)
		<i>Tribolium</i>	<i>Tribolium confusum</i>	Confused flour beetle	Chemical	Benzoquinones	Lis (2011)
		<i>Tribolium</i>	<i>Tribolium castaneum</i>	Red flour beetle	Chemical	Benzoquinones	Lis (2011)
		<i>Ulmoides</i>	<i>Ulmoides (Martianus) o</i>	nd	Chemical	Benzoquinones	Crespo (2011)
		<i>nd</i>	<i>(Palembus) o</i>	nd	Chemical	Ormones	Blum (1994)
		<i>nd</i>	<i>dermesetoides</i>	Beetle	Chemical	Cyanogenetic substances	Blum (1994)
		<i>nd</i>	<i>nd</i>	nd	Chemical	Cyanogenetic substances	Zagobelny (2009)
		<i>nd</i>	<i>Sintomis</i>	nd	Chemical	Toluente	Blum (1994)
Odonata	Cerambycidae	<i>Syllitus</i>	<i>Lytta vesicatoria</i>	Longhorn beetles	Chemical	Chemical	Blum (1994)
		<i>Lytta</i>	<i>Lytta vesicatoria</i>	Spanish fly	Chemical	Chemical	Blum (1994)
		<i>Bruchus</i>	<i>Bruchus lentis</i>	Lentil weevil	Allergic	Cantharidine	Armentia (2006)
		<i>nd</i>	<i>nd</i>	Dragonfly	Parasitical	Phaneropsolus bonnei	Chai (2009)
		<i>nd</i>	<i>nd</i>	Damselfly	Parasitical	Phaneropsolus bonnei	Chai (2009)
		<i>Musca</i>	<i>Musca domestica</i>	Houseflies	Microbial	High bacterial count	Nelson (2006)
		<i>Megaselia</i>	<i>Megaselia scalaris</i>	Humpbacked/ Coffin/Scuttle fly	Parasitical	Miasis	Sehgal and others (2002)
		<i>Diyomiza</i>	<i>Diyomiza formosa</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>Eristalis</i>	<i>Eristalis tenax</i>	Drone fly	Parasitical	Miasis	Sehgal and others (2002)
		<i>Hermetia</i>	<i>Hermetia illucens</i>	Black soldier fly	Parasitical	Miasis	Sehgal and others (2002)
Diptera	Sarcophagidae	<i>Sarcophaga</i>	<i>Sarcophaga peregrina</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
		<i>nd</i>	<i>nd</i>	nd	Parasitical	Miasis	Sehgal and others (2002)
Orthoptera	Gryllidae	<i>Phormia</i>	<i>Phormia regina</i>	Black blow fly	Parasitical	Miasis	Sehgal and others (2002)
		<i>Acheta</i>	<i>Acheta domestica</i>	House cricket	Microbial	High bacterial count	Giaccone and others (2005)
		<i>Sphenarium</i>	<i>nd</i>	Grashopper (chapullines)	Chemical	Lead	Handley and others (2007)
		<i>Triatoma</i>	<i>nd</i>	nd	Parasitical	Chagas disease	WHO (2010); Pereira and others (2010)
		<i>Lophocampa</i>	<i>Lophocampa caryae</i>	Hickory tussock moths	Allergic	Allergic	Piretti and others (1999)
		<i>Gomimbrasia</i>	<i>Imbrasia belina</i>	Mopane worm	Allergic	Allergic	Okezie and others (2010)
		<i>Bombyx</i>	<i>Bombyx mori</i>	Silkworm	Allergic	Allergic	Ji and others (2008)
		<i>Pirallini</i>	<i>Galleria mellonella</i>	Honeycomb moth	Microbial	High bacterial count	Giaccone and others (2005)
		<i>nd</i>	<i>nd</i>	nd	Chemical	Cyanogenetic substances	Blum and others (1994)
		<i>nd</i>	<i>nd</i>	nd	Chemical	Thiaminase	Nishimune and others (2000)
Blattaria	Blattellidae	<i>Anaphe</i>	<i>Anaphe venata</i>	nd	Chemical	Arsenic	Green and others (2001)
		<i>Agrotis</i>	<i>Agrotis infusa</i>	Bogong moth	Chemical	Arsenic	Green and others (2001)
		<i>Periplaneta</i>	<i>Periplaneta americana</i>	Waterbug	Parasitical	Protozoa	Graczyk and others (2005)
		<i>Blattella</i>	<i>Blattella germanica</i>	German cockroach	Parasitical	Protozoa	Graczyk and others (2005)

