

# Insects – a promising feed and food protein source?

Ksenija Nestic<sup>1</sup>, Jutta Zagon<sup>2</sup>

*Abstract:* Insect use in feed and food in European countries is now an increasingly debated topic, although these animals have traditionally been nutritional components in Asian, African, Central American and South American cultures. This review addresses the issue of eating insects and using them as feed components, especially considering the nutritionally important factors. Safety risks are also discussed, as well as allergies, toxicity, consumer acceptance, legislative, welfare and environmental concerns and possibilities for laboratory control. Insects play and will play important roles in the future in various fields of research and utilisation, but especially and increasingly as feed and food ingredients.

**Keywords:** edible insects, entomophagy, insect meal.

## Introduction

It has been estimated that the world population will increase by 25% in the next decades to reach over 9 billion by 2050 (United Nations, 2015). Therefore, a demand to increase food production will be unavoidable, while other influences such as climate change will make this situation more difficult (Nestic, 2018). Recent predictions foresee necessary food augmentation by up to 70% (Hunter *et al.*, 2017), with a particular need for protein sources. In addition, there has been an approximate fivefold increase in global meat consumption since the 1940s as a result of income growth, increasing urbanisation and changes in lifestyles and food preferences. The Food and Agriculture Organization of the United Nations (FAO) forecasted, in a report from 2003, a worldwide increase in meat consumption from 41.3 to 45.3 kg capita<sup>-1</sup> between 2015 and 2030 (FAO, 2003). Meanwhile, recent statistical sources predict much higher figures of global meat consumption, reaching 51.7 kg capita<sup>-1</sup> in 2030 (Statista, 2018). This has also resulted in increasing pressure on the production of protein sources for animal feed. The main protein feedstuffs currently in use are soya and fishmeal, but European countries depend on the import of these ingredients, which makes the livestock sector vulnerable to price inconsistency and trade deviations.

Further deterioration of the situation is the result of the rise in the consumption of fish, as

aquaculture is now the fastest growing food production system in the world. This sector mostly relies on fishmeal as a protein source, but fish farms are increasingly looking to crop proteins as a lower-cost replacement for fishmeal. On the other hand, fishmeal is not only used in aquaculture but is also an excellent source of highly digestible protein ideal for poultry and pig diets. Thus, fish and animal farming are together placing growing demands on protein crops and fishmeal supply. Furthermore, issues regarding the availability of land and water put even more pressure on the world's ability to meet the increasing requirements for the production of animal protein (PROTEINSECT, 2016). The response to this escalating problem goes into two directions: to return to previous strategies or to seek new and alternative solutions.

The first option is based on the reintroduction of processed animal proteins (PAP). Besides being from the fish processing industry, these feedstuffs are largely obtained as by-products of dairies and slaughterhouses. Although in principle bearing nutritional value, for safety reasons they have been excluded from the food chain over the years. PAP eradication from the food chain started after the outbreak of bovine spongiform encephalopathy (BSE) in 1986, when it was found that infectious ruminant prions spread by insufficiently processed meat and bone meal (MBM) used as feed. One of the most important measures was to endorse common European rules to avoid entry of these nutrients

<sup>1</sup>Institute of Veterinary Medicine of Serbia, Autoput 3, 11070 Belgrade, Republic of Serbia;

<sup>2</sup>Bundesinstitut für Risikobewertung (BfR), Max-Dohrn-Str. 8–10 D – 10589 Berlin, Germany.

**Corresponding author:** Ksenija Nestic, [ksenija.nestic@nivs.rs](mailto:ksenija.nestic@nivs.rs)

into the food chain (Nesic and Radosavljevic, 2014). As a consequence, several regulations were established and have been continuously modified, repealed or amended over time according to the findings of regularly updated risk assessments. Still, the basic European Union (EU) Regulation (EC) No. 999/2001 (European Union, 2001) generally prohibits, with a few exemptions, the application of PAP in the human and animal food/feed chains. In addition, regulations (EC) No. 1069/2009 (European Union, 2009a) and (EU) No. 142/2011 (European Union, 2011), prescribe general guidelines for the safe use of animal by-products and provide definitions for the various types of materials of animal origin. Furthermore, the use of protein originating from the same species in animal nutrition was forbidden. Such rigorous measures were introduced due to the fact that those ingredients have potential to cause prion infections not only in animals, but also in human populations consuming food of animal origin. Due to the strict approach during more than 20 years, the epidemiological situation in Europe has improved greatly (Paisley *et al.*, 2008). Even in the United Kingdom, a former hotspot of the BSE crisis, the number of officially reported cases declined to zero in 2016 (OIE, 2018), so the possibility of mitigating the ban opened stepwise. Since 2013, the use of non-ruminant PAP, has been approved again in the EU for use in aquaculture (European Union, 2013). Accordingly, on 1 April 2016, amendments to Serbian Regulation on determination, diagnostics and prevention of transmissible spongiform encephalopathies (Serbia, 2016) were published as a result of harmonisation with EU legislation (Nesic *et al.*, 2016; Nesic and Nikolic-Stajkovic, 2016).

In recent years, insects have become increasingly relevant to satisfy the ever growing need for protein in animal feed, and consequently or directly in the food for humans. As a branch of zoology, entomology has a wide spectrum and is a well developed scientific discipline. It includes the biology and control of insects, as well as their ecology and impact on animal, plant and human health. Although they are often considered a nuisance to human beings and mere pests for crops and animals, insects are crucial components of many ecosystems, where they perform very important functions. They aerate the soil, pollinate blossoms and control insect and plant pests. Many insects, especially beetles, are scavengers, feeding on dead corpses and plants, thereby recycling nutrients back into the soil. Based on this role, forensic entomology was developed as an emerging area in forensic science. It has become an important tool in criminal investigations (Amendt *et al.*, 2011). As decomposers, insects help

create top nutrient-rich layers of soil that help plants grow and, thus, participate in waste bioconversion. Burrowing bugs, such as ants and beetles, dig tunnels that provide channels for water, benefiting plants. Finally, all insects fertilise the soil with the nutrients from their droppings. Some species produce useful substances, such as honey, wax, lacquer or silk, while some have also been used in medicine. Insects are eaten by many amphibians, reptiles, birds and mammals, making their roles in food chains irreplaceable. Also, it is estimated that they form part of the traditional diets of at least 2 billion people and more than 1,900 species have reportedly been used as food (FAO, 2013).

## Insects as feed

The deficit of protein ingredients, especially for animal feed due to the limited availability of natural resources, ongoing climatic changes, food-feed-fuel competition, recent high demand and consequent high prices for fishmeal and soya, increasing production pressure on aquaculture and restrictions on other animal protein usage have led to growing need to include insect protein in diets for aquaculture and livestock. The search for alternative and sustainable proteins is an issue of major importance that requires viable solutions in the short term, making insects an increasingly attractive feed option. Therefore, in mid-2017, the European Commission adopted the amendment EU Regulation No. 2017/893 (European Union, 2017), allowing seven species to be reared and used in feeding aquaculture. This closed list of authorised insects includes: black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domestica*), banded cricket (*Gryllobates sigillatus*) and field cricket (*Gryllus assimilis*). The conditions for the production of insect PAP are strictly regulated. They must be fed only with material of category 3 (material which in principle would still be appropriate for human consumption), not allowing, for example, manure or heavy metal contaminated debris as a feed source. Furthermore, insect PAP has to be treated at least according to method no. 7, following Regulation (EU) No. 142/2011 (European Union, 2011), which means that bacterial contamination must be reduced in order to make a safe product.

The number of new scientific publications on the topic of insects as feed illustrates the growing academic interest, which is also a consequence of the high costs of producing meat, fish and soybean

meal, i.e., around 70% of costs in animal production. Therefore, insects, as good sources of nutrients, having a low environmental impact, requiring less space for production and being already part of the natural diets of pigs, poultry and fish, are an ideal feed alternative (Rumpold and Schluter, 2013). The feed conversion efficiency of insects is good, like crickets for example, which require only 2 kg of feed for every 1 kg of body weight gain (Collavo et al., 2005). Apart from good quality protein, we can also obtain fat as a by-product of protein production, and which can be considered for making biodiesel (Wang et al., 2017).

In the trial of Newton et al. (2005), when a choice was given, pigs did not discriminate against a diet containing ground soldier fly larvae (*Hermetia illucens*) compared with soybean meal. Also, recent experiments on piglets (Spranghers et al., 2018) showed that a substantial amount of soybean products (meal and/or toasted beans) can be replaced with black soldier fly without adverse effects on performance. Furthermore, grasshoppers (*Acrida cinerea*) and Mormon crickets (*Anabrus simplex*) were also able to replace fishmeal and soybean meal in poultry diets (Wang et al., 2007). In several investigations, it was shown that larvae of the common housefly (*Musca domestica*), containing 54% crude protein in dry matter (DM), could successfully replace fishmeal in broiler diets (Teguia et al., 2002; Awoniyi et al., 2004; Hwangbo et al., 2009). When Ijaiya and Eko (2009) replaced 25, 50, 75 or 100 % of fishmeal with silkworm meal in the diet for broilers, they did not find any significant effect on body weight gain, feed intake, feed conversion, slaughtering results or meat quality. In the review of Makkar et al. (2014), 24 feeding experiments (17 from Africa, 4 from Asia and 3 from the USA) with broilers fed various portions of house fly larvae meal (maggot meal) are mentioned. They also included 12 broiler studies (9 from India) describing results with silkworm meal. The authors concluded that fishmeal, soybean meal and groundnut cake could be successfully replaced up to 100 % with insect protein.

Insect protein is a suitable replacement for fishmeal in diets for juvenile fish and crustaceans (Riddick, 2014; van Huis et al., 2015). Insects in the form of meal or pellets can provide adequate protein to replace standard fishmeal in feed for omnivorous fish such as carp and catfish. However, there is evidence that in carnivorous fish such as salmon and trout, only a portion of fishmeal can be replaced with insect products (Riddick, 2014). In spite of that, Lock et al. (2016) found an uneven outcome of fish meal replacement in amounts up to 100% in the diet with two differently processed *H. illucens* meals in

feed for Atlantic Salmon (*Salmo salar*) fed for 15 weeks. The group of fish fed insect meal higher in energy, lipids and carbohydrates performed as well as the fish raised on a fishmeal based diet, whereas fish fed the other insect meal did not. Makkar et al. (2014) reviewed studies with catfish, tilapia, rainbow trout, Atlantic salmon, turbot and crustaceans, where fishmeal was replaced with dried black soldier fly larvae meal, housefly maggots, dried mealworms, locust meal, grasshoppers or silkworm pupae meal. Most studies demonstrated that about 50% replacement of fishmeal had no adverse effect on animal performance. The research of Devic et al. (2018) concluded that inclusions of up to 80 g kg<sup>-1</sup> of black soldier fly larvae meal did not affect the feed quality for advanced nursing tilapia. They emphasised that, as the market price of this ingredient is competitive, feed production costs would be alleviated by reducing use of fish meal, fish oil and soyabean meal. More broadly, inclusions of cheaper, sustainable and locally available feedstuffs in juvenile tilapia commercial feed could support the sustainable intensification of aquaculture and contribute more widely to food security.

Regarding other animal species, insects are used as pet food (e.g. crickets for many insectivorous reptiles and amphibians), and are fed alive or prepared in different ways. The recent opinion of the European Food Safety Authority (EFSA, 2015) noted that some insects are low in calcium content, but they are still suitable in such diets as a natural component for these animal groups.

Anyhow, the policy debate recommends focusing primarily on the use of insects in feed for fish, poultry, pigs and pets, while the use of insects in feed for farmed ruminants like cattle is currently not an issue, although the risk for prion transfection by insects is regarded negligible (EFSA, 2015). The overall positive atmosphere surrounding the idea of using insects in animal feed indicates the situation is favourable for moving forward with this development and taking advantage of this opportunity to improve the real and perceived sustainability of animal feed and livestock production (Verbeke et al., 2015).

## Insects as food

The consumption of insects, or entomophagy, is heavily influenced by cultural and religious practices. These animals, as they belong to the kingdom *Animalia*, are commonly a food source in many regions of the world, especially in Asia, Africa, Central America and South America. In European countries, however, people usually view entomophagy



with disgust and associate eating insects with rather exotic ethnic habits. This attitude has resulted in the neglect of insects in agricultural research for a long time. Despite historical references to the use of insects for food, the topic of entomophagy has only very recently started to attract public attention worldwide (FAO, 2013). Globally, the most commonly consumed insects are beetles (*Coleoptera*) (31 percent), caterpillars (*Lepidoptera*) (18 percent) and bees, wasps and ants (*Hymenoptera*) (14 percent). Following these are grasshoppers, locusts and crickets (*Orthoptera*) (13 percent), cicadas, leafhoppers, planthoppers, scale insects and true bugs (*Hemiptera*) (10 percent), termites (*Isoptera*) (3 percent), dragonflies (*Odonata*) (3 percent), flies (*Diptera*) (2 percent) and other orders (5 percent).

Starting in 2018, Regulation (EU) No. 2015/2283 entered into force, laying down provisions for the approval of novel foods in Europe (European Union, 2015). This new regulation establishes the requirements that enable food business operators to bring new food items onto the EU market, while ensuring high levels of food safety for European consumers. Insects, for which no traditional use in the EU is evident, are explicitly mentioned. Thus, for insects, companies have to make an application which is examined and finally approved or rejected by EFSA. However, in certain cases a simpler notification procedure is possible for traditional food from third countries if it is proven that such foods have been part of human nutrition for at least 25 years without any safety concerns. Accordingly, it is reasonable to expect that more insects and insect products will enter the European food market. Interestingly, in Switzerland, an EU-associated partner state, since 1 May 2017, three insect species (*Acheta domesticus*, *Locusta migratoria* and *Tenebrio molitor*) have already been allowed for human consumption. Nevertheless, up to now, there are no systematically collected data available on insect consumption in Europe, although it is known they are used in some elite cuisines, but not comparable with their use in North-Eastern India, South-East Asian countries and parts of Australia. Premalatha *et al.* (2011) gave some examples: wasps, bamboo caterpillars, cricket and locusts are sold as delicacies in the finest restaurants and food shops in Thailand; annual sales of ant food in China reach \$100 million; the rice field grasshopper, called inago, is a luxury food item in Japan; canned wasps, as a 65 g can, sell for over \$10, while hornets are even more expensive, selling at over \$20 per 100 g. There is an explosion of tourist interest in the native Australian “bush tucker” foods, which include insects such as wickety grubs (*Cossidae*), bogong

moth and bardee larva. In Mexico, upmarket restaurants charge upwards of \$25 per plate of escamoles (pupae of an ant species) and gusanos (butterfly larvae), and when exported to Canada, escamoles must fetch a fantastic price of \$50 for a 30 g can (almost \$2 per gram).

Previously, insects were eaten alive and later, in some cultures, they were also served cooked, roasted or boiled (either insects *per se* or insect additives to food), or prepared using other culinary techniques. Many people prefer incorporating insects into the food in a manner that renders them not visible – only accepting the idea that insects have beneficial nutritional value. This shows that people, especially in North America and Europe, would eat insects if they do not know what they are eating, with the exception of individuals who have allergic reactions (and are aware of possible cross reactions) (Mlcek *et al.*, 2014). These factors suggest that insect transformation, specifically their mode of processing, most certainly would facilitate consumption in the future. In practice, dried insects can be crushed or pulverised, and raw or boiled insects ground or mashed, making their form unrecognisable. Extract of insect protein and fat can also be used. If insects are prepared as masses of protein and lipids, these can be mixed with other foodstuffs, such as grain, ground meat or mashed potatoes to make a variety of dishes and become more acceptable to most people. Insects can also be a serious alternative for vegetarians and – though at present rather hypothetical science fiction – their nutritional potential for a relatively long-term future human settlement in space has been discussed (Mlcek *et al.*, 2014).

Among different possibilities for preservation and storage of insects, the most appropriate have to be chosen. Live insects, after washing, are typically transported in ice coolers shortly after collection. Refrigeration is also recommended for fried and boiled insects. Some are preserved and traded after sun-drying. Other simple preservation methods such as acidifying insects with vinegar have been successful. Another example is the use of insects for protein enrichment in fermented food products. This is a viable processing option with mutual benefits, since the decreased pH in lactic acid-fermented products prevents the growth of potentially harmful microorganisms (Klunder *et al.*, 2012). Sometimes freeze-drying is implemented. However, other contemporary preservation methods should be explored, such as the application of ultraviolet light and high-pressure technologies, as well as adequate packaging methods. For this entire topic, further research on the best, most suitable solutions is necessary.

**Nutritional value**

Insects are usually considered as valuable sources of protein. Because of their high protein content, well balanced amino acid composition and the other nutrients they contain, beside their use as animal feed, they could become an attractive alternative to traditional foods of animal origin, such as milk, meat, fish and eggs in human nutrition. Depending on the insect species, protein levels can be up to 63% on a DM basis, as is the case with the larvae of black soldier fly (*Hermetia illucens*) and house fly (*Musca domestica*), which contain up to around 63% protein and 36% fat (Makkar et al., 2014). They contain high levels of key amino acids (e.g. lysine, tryptophan) when compared to most crop plants. A recent study comparing nutritive characteristics of a range of insects showed that the amino acid profile of dipteran insects is superior to soybean meal and more similar to fishmeal (Barroso et al., 2014).

Lipids are a large component of fly larvae with crude fat content in meal produced from house fly larvae reported to range from 14 to 27% (Fasakin et al., 2003; Aniebo et al., 2008; Pretorius, 2011). The principal fatty acids found in *M. domestica* larvae

and pupae are palmitic, palmitoleic, oleic and linoleic (St-Hilaire et al., 2007; Hwangbo et al., 2009).

Chitin comprises the main carbohydrate in insects. As reviewed by Mlcek et al. (2014), the carbohydrate content of edible insects ranged from 6.71% to 15.98%. Studies dealing with the vitamin content in insects are insufficient, although it is known that edible insects contain mainly carotene and vitamins B1, B2, B6, D, E, K and C. Analysis of mineral elements showed that edible insects are rich in nutritious elements such as potassium and sodium (e.g. cricket nymph), calcium (e.g. cricket adult), copper (e.g. *Usta terpsichore*, mealworm adult), iron (e.g. axayacatl – a mixture of several species of aquatic Hemiptera, giant mealworm), zinc (e.g. cricket nymph), manganese (e.g. cricket adult) and phosphorus (e.g. cricket adult). Summarised proximate analysis data by Makkar et al. (2014) and EFSA (2015) on crude nutrients in different insect species are shown in Table 1.

Tables 2 and 3 show some collected data on nutritional composition of insects in comparison to other food protein sources.

As Premalatha et al. (2011) noted, it is a supreme irony that all over the world billions are spent every year to save crops that contain no more than

**Table 1.** Crude nutrients of different insect species measured by Weende analysis, in % of dry matter (Adapted from Makkar et al. (2011) and EFSA (2015))

Insect species	Crude protein %	Crude fat %	Carbohydrates %	Crude ash %
Black soldier fly larvae	41.1–43.6	15.0–34.8	7.0	14.6–26.8
Housefly maggot meal	42.3–60.4	9.0–26.0	1.6–8.6	6.2–17.3
Tenebrio molitor	47.2–60.3	31.1–43.1	7.4–15.0	1.0–4.5
Locust or grasshopper meal	29.2–65.9	4.2–14.1	2.4–14.0	4.4–10.0
House cricket	55.0–67.2	9.8–22.4	15.7–22.1	3.6–9.1
Silkworm pupae meal	51.6–70.6	6.2–37.1	2.5–5.8	3.3–10.6

**Table 2.** B-vitamins in 100 g servings of chicken and bean dishes in comparison to the content of some insects (Adapted from Premalatha et al. (2011)).

	Thiamine	Riboflavin	Niacin
Daily human requirement	1.5 mg	1.7 mg	20 mg
Portion from roasted chicken	5.4%	–	45%
Portion from backed beans	10.8%	–	3%
Portion from termites	8.7%	67.4%	47.7%
Portion from silkworm larvae	224.7%	112.2%	26%
Portion from palm weevil	201.3%	131.7%	38.9%

**Table 3.** Protein and iron in 100 g servings of beef and of two insects (Adopted from Premalatha *et al.* (2011)).

Food	Protein, g	Iron, mg
Beef (boiled)	22.3	2.9
Silkmoth larvae (boiled)	28.2	35.5
Grasshoppers (fried)	61.1	–

14% of plant protein by killing another food source (insects) that can contain up to 75% of high quality animal protein.

### What bugs us about edible bugs?

A major consideration in the use or applicability of any novel food product is to demonstrate its safety. Studies on the microbiological and chemical safety of insects reared for feed and food are limited, but some authors have published review papers on this issue (Belluco *et al.*, 2013; Van der Spiegel *et al.*, 2013), FAO discussed it in its booklet (FAO, 2013), EFSA gave summarised data in its scientific opinion (EFSA, 2015) and project PROTEINSECTS reported their final viewpoint (PROTEINSECTS, 2016). Finally, all of them agree that insects as a feed and food category, in principle, could be consumed with no additional hazards in comparison with usually eaten animal products. Levels of more than 500 potentially toxic chemical contaminants were all below recommended maximum amounts suggested by bodies such as the European Commission, World Health Organisation and Codex. However, elevated levels of the toxic heavy metal cadmium in some insects indicated that pre-screening of rearing substrates would be necessary to mitigate risk. Tests found no evidence of the presence of viable biological contaminants such as *Salmonella* and *Campylobacter* (PROTEINSECTS, 2016). On the basis of these facts, insects can be regarded as safe, if properly managed and consumed.

Some toxicological and allergy threats were also considered. Relatively heat-resistant thiaminase was detected and characterised from the silkworm, so it needs thorough heat treatment for detoxification (Nishimune *et al.*, 2000). Pesticide applications against locusts and grasshoppers can cause problems because of their toxic residues (Van Huis, 2003; Yen, 2009). In most cases, allergies to insects are associated with a job where employees deal with insects. In clinical practice in relation to insects, the most commonly reported allergic reactions are to chitin, which is the second most abundant biopolymer in nature (Rop *et al.*, 2009). Crickets can trigger allergic

reactions in sensitive consumers (Fernandez-Cassi *et al.*, 2018). Homologue proteins shared between different species can cause such responses. Tropomyosin, arginine-kinase and glyceraldehyde-3-phosphate dehydrogenase have been identified as highly allergenic. Hexamerin B1, with allergenic potential requiring more research, has been described as a specific cricket allergen. Pilot results of Broekman *et al.* (2016) suggest that shrimp-allergic patients might be at risk for mealworm allergy because IgE binding to tropomyosin and arginine kinase (major shellfish allergens) and arcoplasmic calcium-binding protein and myosin light chain (minor shellfish allergens) were detected. For safety reasons, insect food products should be labelled to raise awareness in susceptible consumers.

Consumer acceptance is the key to the successful adoption of insects as a source of protein for feed and food. Recent studies have shown that willingness to eat insect based foods is determined by a diversity of personal attitudes and interests (food neophobia i.e., fear of trying new foods; interest in the environmental impact of personal food choices and; openness to change dietary habits), as well as cultural exposure, familiarity or past experience and knowledge (Verbeke, 2015; Tan *et al.*, 2015). One of the most common refusals is based on hygienic reasons, but most insects, especially edible insects such as grasshoppers and lepidopteran or coleopteran larvae mostly eat fresh plant leaves or wood and are, therefore, cleaner and more hygienic than crabs or lobsters, which eat carrion. In general, the application of insects for food and feed purposes is environmentally more beneficial than traditional sources of proteins (Smetana *et al.*, 2016). Insects can be reared on organic side-streams (including human and animal waste), emit fewer greenhouse gases and less ammonia than cattle or pigs and require significantly less land and water than cattle rearing. As public attention could gradually evolve over time, willingness to adopt the use of insects as nutritive dietary components can modify. Especially, their implementation in animal feed can be expected to evolve.

Other issues such as the welfare of insects raised for use in feed and food also need to be considered. Harvesting insects as food from the wild,

like any other hunting and collecting activities, has the potential to become a threat to both the target species and the environment. Ramos-Elorduy (2006), for example, reported that the populations of some of the 30 edible insect species in the Mexican town of Tulancingo have declined because of over-exploitation, and this situation has led to a call for regulation of edible insects' exploitation in Mexico to ensure better management, production and conservation. Semi-cultivation of edible insects is often used, as well as rearing and farming in various degrees. To ensure animal welfare, farmed insects should be provided with adequate space to minimise mortality and increase productivity. Little is known about the extent to which insects experience pain and discomfort (Erens et al., 2012), but insect-killing methods that would reduce suffering include freezing or instantaneous techniques such as shredding.

Further regulatory aspects have to be solved in the future. Western legislation is greatly focused on safety concerns about new food or new ingredients (Belluco et al., 2013). In addition, there are merely technical difficulties, e.g., regarding slaughterhouse registration, which is not yet applicable to insects, and in relation to the substrate on which the insects are reared. Insects produced for feed would be classed as farmed animals, for which only category 3 materials are allowed as a feed source. Manure is a category 2 material and, therefore, not permitted to be fed to farmed animals; neither can insects be fed on catering waste or former foodstuffs containing meat and/or fish.

As for any other ingredient, adequate monitoring and control of feed and food has to be implemented. However, no laboratory test for their detection in feed/food has been officially validated and standardised yet.

## Laboratory control

Enforcement of any legislation requires adequate analytical tools and implementation of an appropriate control system (Nesic and Pavlovic, 2012). Hence, the design of laboratory methods should serve for monitoring authenticity, control of labels/declarations and detection of fraud (van Raamsdonk et al., 2017). When it comes to insects that belong to animal protein and are subject to PAP regulations, the only methods authorised for feed testing are light microscopy and polymerase chain reaction (PCR) (European Union, 2013). There is also a corresponding harmonised regulation in Serbia (Serbia, 2016). These two methods are used for aquaculture

feed control individually or in combination (EURL-AP, 2015), but usually microscopy gives the first information on the presence of PAP material. Light microscopy can distinguish between fish and terrestrial particles, as it relies principally on the categorisation of bone fragments into those two groups. The team of the EU Reference Laboratory for animal proteins in feedstuffs recently proposed an improvement of Annex VI of EU regulation (EC) No. 152/2009 (European Union, 2009b), publishing a modified and adapted microscopy protocol for detection of insects. Furthermore, the implementation of a third category of animal material in addition to terrestrial as "terrestrial invertebrates" was proposed (Veys et al., 2018).

However, microscopic discrimination between authorised species and undesired ones is not possible. Hence, the use of other methods, such as DNA targeting of authorised species, would offer additional information. Polymerase chain reaction (PCR) is the most frequently applied method for reliable detection of species-specific DNA. It has already been officially accepted for ruminant DNA detection in feed (EURL-AP, 2017), and two new protocols for poultry and pig DNA have been validated by the European Union Reference Laboratory for Animal Proteins in Feedingstuffs, but not yet included in the official catalogue of methods. Regarding other animal species detection, PCR has also found a role especially in forensic investigations (Davitkov et al., 2017), while forensic entomology as a scientific discipline developed the application of insects in criminal investigation (Joseph et al., 2011). To unravel food fraud, PCR is the most promising method of choice (Nesic et al., 2017).

To fill the existing analytical gaps and to detect the species of interest rapidly, the most promising approach today is real-time PCR. Recently, three fully validated methods were published. One protocol is to detect in food and feed the beetle *Tenebrio molitor* (Debode et al., 2017), the larvae of which are allowed for aquaculture feed, but are also a popular pet food for reptiles and birds. Two papers were published on the detection of black soldier fly (*Hermetia illucens*) in feedstuffs by newly developed real-time PCR methods (Marien et al., 2018; Zagon et al., 2018). However, five other insect species are allowed in feed and for which proper detection and control methods remain to be ascertained, while more than one million species of insects are unable to be discriminated. There is also a challenge in relation to food matrices and, as mentioned by Zagon et al. (2018), it might be expected that sooner or later, insects will be added to the list of commodities in the frame of the European allergen labelling



directives. To cope with the huge diversity of insects belonging to the big phylum of arthropods, the possibility of multiplexing solutions with other insect-targeted PCR methods could be an option. Alternatively, the application of next-generation sequencing (NGS) based on metabarcoding of arthropods (Deiner *et al.*, 2017; Richardson *et al.*, 2018; Toju and Baba; 2018) can be considered. This powerful technique enables the simultaneous detection of many different species in one and the same DNA extract, as demonstrated for native DNA isolated from environmental samples or faeces from insectivorous animals to evaluate their spectra of insect prey (Sint *et al.*, 2014; Galan *et al.*, 2018). A major challenge in terms of processed materials will be to identify universal primers targeting DNA fragments of suitable size ( $\leq 150$  bp), but at the same time covering all major groups of insects which are at least *Diptera*, *Orthoptera*, *Lepidoptera* and *Coleoptera*, accounting for the most important cultured insects. Apart from this, sensitivity in the case of only trace amounts of one particular species might be an issue, as could be economic aspects, since NGS machines, consumables and operator's knowledge are significant costs for these tests. The future will show how far and how rapidly this new sequencing method will find its way into routine application.

In any case, DNA-based methods and possibly some other laboratory tests used in feed control, like immunoassays (Nesic *et al.*, 2012), near-infrared spectrometry or mass spectrometry, at present, should probably be used as complementary tests once insect fragments have been microscopically confirmed in a non-specific screening approach (Nesic *et al.*, 2014). This would be a continuation of the present strategy for the disclosure of PAPs in feed (Veys *et al.*, 2018; EFSA, 2018). Nevertheless, different teams worldwide are rapidly pursuing research work and results are expected soon, as feed regulations in this area certainly will reflect on and push forward the science. Surely, food control, in response to future regulative tendencies, will require analytical approaches at the highest level.

Although up to now, no thresholds for PAPs have been implemented, it has to be critically remarked that still no reliable quantitative method exists. DNA-based methods (qPCR) are well suited for relative quantitation (e.g. to express the percent DNA of a given species in relation to DNA of another matrix component), as, for example, in genetically modified organism (GMO) detection. This

is easily done if single copy genes are chosen for quantitation, and standard matrices or plasmids are available. Absolute results can be expressed as copy numbers of the DNA fragment under investigation. Further, digital PCR techniques, determining exact copy numbers by separation of DNA targets in multifold reactions, are ideally suited for copy number-based relative quantitation, e.g., as proven for GMO (Koeppel and Bucher, 2015). However, if absolute amounts (gram) of an analyte are to be calculated, the true value is difficult to deduce merely from copy numbers, since the amount of DNA in different tissue types can vary considerably. The bias is worse if, to enhance sensitivity, multi-copy genes (e.g. mitochondrial genes) are targeted by the PCR method. Nonetheless, encouraging results were achieved in estimating the prey-predator relation for sea lions using multi-copy targets in qPCR for species detection in scats (Tollit *et al.*, 2009). This principle might also be an option for discriminating allowed insect species against the background of unknown insect species. However, such quantitative approaches require well-characterised, universal group-specific primers and possibly numerical and empirically evaluated correction factors. Therefore, intensive research in this field is urgently required.

## Conclusion

Based on novel data, insects represent a promising feed and food protein source, but future research needs to provide some solutions before they can be widely utilised in food and/or feed. Clarification is required to determine how the nutritional value of insects can be managed systematically, establish clear processing and storage methodologies, define rearing practices and implement regulations and adequate laboratory control with regard to food and feed safety. Overall, entomophagy can be promoted for several reasons: insects, if reared properly, are in principle, healthy, sustainable and nutritious alternatives to other animal food/feed sources, while insects' low negative environmental influence favours them, and economic factors are also on their side. Last but not least, the decision should be up to the informed consumer, accompanied by thorough risk assessment and correct labelling and controls, whether to accept this kind of novel food and feed in the future.



# Insekti – potencijalni izvor proteina?

Ksenija Nešić Jutta Zagon

**Abstract:** Mogućnost upotrebe insekata kao izvora proteina u hrani i hrani za životinje tema je o kojoj se sve više raspravlja u evropskim zemljama, mada su ove životinje tradicionalno korišćene u ishrani u azijskim, afričkim, srednjeameričkim i južnoameričkim kulturama. U radu je predstavljan pregled primene insekata u nutritivne svrhe, a takođe su navedeni potencijalni rizici po bezbednost, alergijski aspekti, toksičnost, prihvatljivost za potrošače, zakonodavstvo, elementi dobrobiti, uticaj na životnu sredinu i mogućnosti za laboratorijsku kontrolu. Insekti imaju mnogobrojne važne uloge u različitim oblastima istraživanja i upotrebe, ali se sve više pominju kao hraniva i prehrambeni sastojci budućnosti.

**Key words:** entomofagija, jestivi insekti, hrana i hrana za životinje.

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