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INSECTS – A NATURAL NUTRIENT SOURCE FOR POULTRY – A REVIEW*

Damian Józefiak[1♦], Agata Józefiak[3], Bartosz Kierończyk[1], Mateusz Rawski[1], Sylwester Świątkiewicz[4], Jakub Długosz[1], Ricarda Margarete Engberg[2]

[1]Department of Animal Nutrition and Feed Management, Poznan University of Life Sciences, Wołyńska 33, 60-637 Poznań, Poland
[2]Department of Animal Science, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark
[3]Veterinary Institute, Poznan University of Life Sciences, Wołyńska 35, 60-637 Poznań, Poland
[4]Department of Animal Nutrition and Feed Science, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland
[♦]Corresponding author: damjo@up.poznan.pl

Abstract

The consumption of poultry meat and eggs is expected to increase considerably in the nearest future, which creates the demand for new poultry feed ingredients in order to support sustainable intensive production. Moreover, the constant improvement of the genetic potential of poultry has resulted in an increased nutrient density in poultry feeds, which limits the possibility to include low quality feed ingredients. Therefore, the feed industry needs new sources of highly digestible protein with a desirable amino acid composition to substitute other valuable but limited protein sources of animal origin, such as fishmeal. With estimated 1.5 to 3 million species, the class of insects harbours the largest species variety in the world including species providing a high protein and sulphur amino acids content, which can be successfully exploited as feed for poultry. The aim of this paper is to review the present state of knowledge concerning the use of insect protein in poultry nutrition and the possibilities of mass production of insects for the feed industry. There is no doubt that insects have an enormous potential as a source of nutrients (protein) and active substances (polyunsaturated fatty acids, antimicrobial peptides) for poultry. It can be concluded, based on many experimental results, that meals from insects being members of the orders Diptera (black soldier fly, housefly), Coleoptera (mealworms) and Orthoptera (grasshoppers, locust, crickets and katylids), may be successfully used as feed material in poultry diets. However, legislation barriers in the European Union, as well as relatively high costs and limited quantity of produced insects are restrictions in the large-scale use of insect meals in poultry nutrition.

Key words: insects, poultry, protein, antimicrobial peptides

The requirement of valuable protein sources for a continuously growing human population and the simultaneous decrease of available areas suitable for agricultural

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production present a serious future global challenge. In this context, the global demand for poultry meat and eggs is expected to increase significantly, which is due to the fact that these products have a very high nutritional value, they are relatively cheap and no religious issues are connected with their consumption. Furthermore, when compared to other livestock, poultry production is considered to be relatively environmentally friendly having a significantly lower CO_2 footprint. The increasing intensity of poultry production requires higher amounts of protein to cover the amino acid requirements for plumage development, growth and egg production (Hossain and Blair, 2007). Currently available vegetable protein sources for poultry include soybean meal, rapeseed meal, legumes, and different cereal by-products. However, the amino acids composition of plant proteins for poultry is inferior to that of animal based proteins, specifically with respect to their content of essential sulphur containing amino acids, in particular methionine. Therefore, fishmeal is still quite commonly used in poultry diets. However, due to overfishing, fishmeal has become a very limited resource which is reflected by increasing market prices over the last decades. Alternative protein sources of comparable value are therefore urgently needed in order to make poultry production a sustainable production form in the future. Due to the reasons mentioned above, the potential of insect protein in poultry diets has attracted much attention. Chickens with access to outdoor areas pick up insects at all life stages and eat them voluntarily, which indicates that they are evolutionarily adapted to insects as a natural part of their diet (Bovera et al., 2015). Therefore, it seems reasonable to consider the inclusion of insect proteins as raw material to be used in commercial feed manufacturing and to develop intensive farming systems for new six-legged livestock. In order to ensure cost effective insect based protein production, the ideal insect candidate should have a short reproduction cycle and should be nutritious, providing high concentrations of protein and sulphur containing amino acids. In order to guarantee a constant insect supply, the ideal insect candidate should further be easy to rear in intensive production sites (Hossain and Blair, 2007).

Nutrient composition of insects

Insects at all life stages are rich sources of animal protein (Bovera et al., 2015). Until now, the main research efforts have focussed on the mealworm (larvae of the beetle *Tenebrio molitor),* the maggot and pupae of the housefly (*Musca domestica*), the black soldier fly (*Hermetia illucens*), and insect families belonging to the order Orthoptera including locusts, grasshoppers, crickets and katylids. However, insects of the order Blattodea, like American (*Periplaneta americana*), German (*Blattella germanica*), and Asian (*Blattella asahinai*) cockroaches are also interesting candidates (Helm et al., 1990). A comprehensive review on the nutrient composition of different insects and their meals including larvae of the black soldier fly, housefly maggot and pupae, mealworms, as well as locusts, grasshoppers, crickets, and silkworm pupae is provided by Makkar et al. (2014) and Sánchez-Muros et al. (2014).

Table 1. Amino acid composition of different insects and fish meal in % of crude protein (Van Broekhoven et al., 2015; De Marco et al., 2015; Makkar et al., 2014; Józefiak, unpublished)

	Tenebrio molitor larvae	*Gryllodes silligatus* subimago	*Gryllus assimilis* imago	*Hermetia illucens* larvae	*Shelfordella lateralis* subimago	*Musca domestica* larvae	Fishmeal
Amino acid	% of crude protein						
Histidine	2.7	2.2	2.1	2.6	2.5	2.8	2.6
Arginine	4.5	5.7	5.8	4.8	5.6	4.9	5.8
Threonine	3.6	3.5	3.3	3.6	3.3	3.3	4.3
Tyrosine	5.4	4.2	4.5	6.0	5.6	5.1	3.1
Valine	5.9	5.2	5.3	5.6	5.1	4.4	4.8
Methionine	1.2	1.6	1.2	1.4	1.3	2.2	2.9
Cysteine	0.6	0.9	0.5	0.7	0.7	0.4	1.2
Isoleucine	4.0	3.7	3.4	4.0	3.1	3.2	4.0
Leucine	6.9	6.9	6.6	6.6	5.8	5.7	7.4
Phenylalanine	3.2	3.1	2.9	3.8	3.0	5.0	3.6
Lysine	4.9	5.3	5.0	5.6	4.9	6.9	7.8
Tryptophan	1.0	0.9	0.7	1.1	0.8	3.2	1.2
Total	43.9	43.2	41.3	45.8	41.7	47.1	48.7

Table 2. Amino acid composition of different insects and fish meal in % relative to lysine (Van Broekhoven et al., 2015; De Marco et al., 2015; Makkar et al., 2014; Józefiak, unpublished)

	Tenebrio molitor larvae	*Gryllodes silligatus* subimago	*Gryllus assimilis* imago	*Hermetia illucens* larvae	*Shelfordella lateralis* subimago	*Musca domestica* larvae	Fishmeal
Amino acid	% relative to lysine						
Lysine	100	100	100	100	100	100	100
Histidine	55	42	42	46	51	41	33
Arginine	92	108	116	86	114	71	74
Threonine	73	66	66	64	67	48	55
Tyrosine	110	79	90	107	114	74	40
Valine	120	98	106	100	104	64	62
Methionine	24	30	24	25	27	32	37
Cysteine	12	17	10	13	14	6	15
Isoleucine	82	70	68	71	63	46	51
Leucine	141	130	132	118	118	83	95
Phenylalanine	65	58	58	68	61	72	46
Tryptophan	20	17	15	20	17	46	15

Table 3. Nutrient composition of different insect meals (Van Broekhoven et al., 2015; De Marco et al., 2015; Makkar et al., 2014; Józefiak, unpublished)

Item	*Gryllus assimilis*	*Musca domestica*		*Hermetia illucens*	*Tenebrio molitor*		*Blatta lateralis*
	imago	subimago	pupae	larvae	larvae	larvae	nymph
Per kg DM							
gross energy (MJ)	21.5	19.3	20.1	20–24	22.1	26.8–27.3	-
crude fibre (g)	70	94	157	16–86	70	51–88	86–89
ash (g)	64	54	55–98	31–173	146-284	10–45	46–54
phosphorus (g)	8.0	8.6	-	9.2–24.0	6.4–15.0	4.4–14.2	0.6–0.7
calcium (g)	9.9	3.1	-	3.1–8.0	50.0–86.0	0.3–6.2	0.2
crude protein (g)	564	638	630–762	380–604	411–450	451–603	543–734
crude fat (g)	238	168	144–161	90–260	150–350	250–431	176–261
Amino acids per 16 g N							
glycine (g)	3.0	2.7	3.9–4.3	3.7–5.1	5.1	3.9–5.6	4.6–4.8
arginine (g)	3.7	3.3	4.2–5.9	3.7–5.8	4.8–8.0	3.8–5.6	3.8–5.6
threonine (g)	2.1	1.9	3.0–3.4	2.0–4.4	1.3–4.8	3.5–4.4	2.5–3.3
valine (g)	3.4	3.0	3.4–4.6	1.3–5.1	5.6–9.1	5.5–6.6	4.4–5.1
methionine (g)	0.8	0.8	1.5–2.6	1.3–4.6	1.4–2.4	1.1–2.0	1.1–1.2
cysteine (g)	-	-	0.4	0.5–1.0	0.1	0.8–0.9	-
leucine (g)	4.2	3.6	4.9–5.4	4.5–7.8	6.6–8.4	6.7–10.6	4.7–5.8
lysine (g)	3.2	2.9	4.8–6.5	5.0–8.2	5.5–8.0	4.6–6.1	4.0–4.9
Fatty acids per kg fat							
total SFAs (g)	351	352	476	417	749	229–334	28.7
total MUFAs (g)	298	261	307	314	155	407–536	46.3–50.2
total PUFAs (g)	336	369	291	399	74	254–323	138–219
total PUFA *n-3* (g)	22	17	-	-	2	2–4	1–11
total PUFA *n-6* (g)	314	352	-	-	23	81–93	35–207

Insects are a rich source of protein, essential amino acids and fat (Tables 1, 2 and 3) (Józefiak, unpublished; Van Broekhoven et al., 2015; De Marco et al., 2015; Makkar et al., 2014). The protein content of insect meals varies considerably from around 40% up to 60% even when the meals are based on the same insect species (Table 3). The same holds true for the fat content. However, it is important to note that insect meals compared to fishmeal contain lower concentration of methionine, which has to be considered when formulating diets based on insect proteins. Further, the calcium concentration is usually lower than that of fishmeal (Józefiak, unpublished: Van Broekhoven et al., 2015; De Marco et al., 2015; Makkar et al., 2014). Larvae of the black soldier fly provide substantially more calcium (Table 3) than other insects (De Marco et al., 2015; Józefiak, unpublished). The nutrient concentration of insects depends on their life stage as well as the rearing conditions and the composition of the growth media used for insect production (Makkar et al., 2014). For instance, house fly larvae grown on chicken manure had a lower dry matter (DM) content, but a higher methionine content per kg of DM as compared to larvae grown on a medium containing wheat bran, alfalfa, malt and dried yeast (Engberg et al., unpublished). Mealworm larvae prefer protein based diets rather than starch based diets and they appreciate the inclusion of yeast (van Broekhoven et al., 2015). The feed conversion ratio of mealworms fed 11.9% crude protein was 6.05 kg/kg and improved significantly to 3.04 kg/kg when feed with a crude protein content of 32.7% was fed (van Broekhoven et al., 2015). No significant change in protein concentration of the mealworm meal was observed, however, the fat content of mealworms fed low protein diets was significantly lower as compared to high protein diets (18.9 vs. 26.3 %) (van Broekhoven et al., 2015).

The high water content of live insects (average of 30% dry matter) may cause problems in commercial feed milling operations and it is important to standardize the processing conditions for meal production to obtain high-quality raw material. At present, no literature is available that would provide information on the effect of processing on the protein quality of insect meal. However, crude protein and crude fat are the main nutrients in dried insect meal. Therefore, similarly to other meals of animal origin, oxidation processes and microbial deterioration during storage (Awoniyi et al., 2004) determine the shelf life quality and should be taken into consideration.

Chitin in its pure form is the most widely occurring polysaccharide in nature and is found in the cuticle of crustaceans and insects, in many other invertebrates, in nematode eggs, and as a structural cell wall component of fungi. Usually it is found as complex compound composed of chitin combined with cuticular proteins, lipids and minerals, e.g. calcium (Kramer et al., 1995; Nation, 2008). Most insects contain only minimal amounts of micro and macroelements in their cuticle (Johnson and Peniston, 1982; No et al., 1989), however, some species such as pupae of the face fly (*Musca autumnalis*) and larvae of the black soldier fly (*Hermetia illucens*) contain a significant amount of calcium in their cuticle (Dashefsky et al., 1976; Roseland et al., 1985; Tomberlin et al., 2002). The acid detergent fibre (ADF) and crude fibre (CF) analyses can be applied as indicative methods to evaluate the chitin concentration present in the insect cuticle. Currently, only little data is available regarding the content of chitin in whole insects based on enzyme assays, which may be a more accurate method of determining the amount of this component. Based on these analyses, Cauchie (2002) found that the larvae of aquatic insects (Coleoptera, Dictyoptera, Diptera, Ephemeroptera) contain between 2.7–16.2% of chitin, in DM (Figure 1). Klasing (1998) found that the content of chitin in arthropods ranges from 18 to 60%.

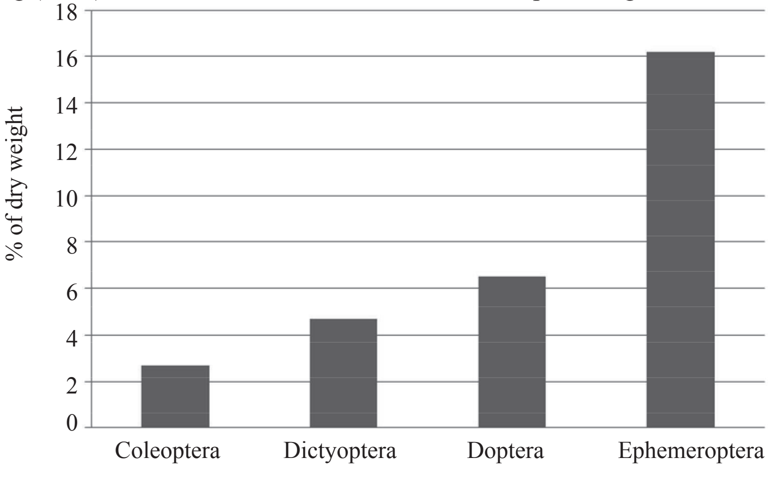

Figure 1. Chitin content of insects (% of dry weight) (Cauchie, 2002)

Insect antimicrobial peptides – added value?

In recent years, much attention has been devoted to antimicrobial peptides (AMPs), called natural antibiotics. Intensive research is being conducted on the possibility of using these compounds in agriculture, including animal nutrition, as well as the pharmaceutical industry. In general, it is believed that their activity does not lead to the development of bacterial resistance. Insects are a rich source of AMPs. Most insect AMPs are small, cationic proteins which exhibit activity against bacteria and/or fungi, as well as certain parasites and viruses.

The antibacterial effect of AMPs on the bacterial cell involves the destruction of the bacterial cell envelope. AMPs are cationic peptides which bind to and interact with negatively charged cell membrane lipids including anionic phospholipids and phosphate groups of lipopolysaccharide of Gram-negative bacteria, as well as teichoic and lipoteichoic acids composing the peptidoglycan layer of Gram-positive bacteria. When the peptide anchors in the cytoplasmic membrane of the microorganism, a change in the membrane structure occurs, resulting in the incorporation into the phospholipid dual layer of the cytoplasmic membrane. Penetration of AMPs inside a cell may affect the nucleic acid and protein synthesis which may explain their activity in the case of antibiotic-resistant microorganisms (Żyłowska et al., 2011). The largest group of insect AMPs are defensins. Insect defensins are peptides consisting of 34–51 residues with six conserved cysteines. Defensins have been identified in numerous insect species belonging to the orders Diptera, Hymenoptera, Coleoptera, Lepidoptera, Hemiptera, Isoptera, Odonata (Yi et al., 2014). Currently, we know about 170 defensins present in invertebrates. These peptides are produced by body fat cells, as well as blood cells – thrombocytes, from where they can be easily diffused and act throughout the whole body. Insect hemolymph gains antimicrobial properties after the insect is wounded or after microbial induction. Insect defensins are active mainly against Gram-positive bacteria, including *Micrococcus luteus, Aerococcus viridians, Bacillus megaterium, Bacillus subtilis, Bacillus thuringiensis*, and *Staphylococcus aureus*. Some insect defensins are also active against Gram-negative bacteria, *Escherichia coli* (Lee et al. 2004; Lowenberger et al., 1995; Seufi et al., 2011; Ueda et al., 2005; Vizioli et al., 2001; Yamada and Natori, 1993). Further, antifungal properties have been observed in some insect AMPs, e.g. termicin in *Pseudacanthotermes spiniger*, drosomicin in *Drosophila melanogaster,* heliomicin in *Heliothis verescens* and gallerimicin in the pupae of *Galleria mellonella* (Aerts et al., 2008; Żyłowska et al., 2011). Little is known about the activity of defensins and defensin-like peptides of *Hermetia illucens*. A recent study identifies a novel AMP – defensin-like peptide 4 (DLP4) from *Hermetia illucens* (Park et al., 2005). This protein displays antimicrobial properties against primarily Gram-positive bacteria, e.g. MRSA, *S. aureus* 40881, *S. aureus* 12256, *S. epidermidis* and *Bacillus subtilis*. Real-time PCR analysis of the expression of DLP4 in different tissue of the black soldier fly showed the highest expression in the trachea and fat body (Park et al., 2015).

Proteins with antimicrobial activity have also been extracted from the larvae of *Tenebrio molitor.* Examples are different tenecins which are active against Gram-positive bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus pyrogen, Micrococcus luteus, Corynebacterium diphtheriae* but also against fungi.

Insect antimicrobial peptides provide great hope due to the increasing global problem of bacterial resistance to antibiotics. The antimicrobial mechanism of insect AMPs is shaped over many years of evolution and it is very conservative, which in practice means that, opposite to antibiotics that quickly induce bacterial resistance, these component may remain effective against bacteria.

Insect protein in poultry production

Plant derived protein is a key ingredient of farm animal feed around the world. However, in many cases it contains low amounts of lysine, tryptophan, threonine and methionine (Bukkens, 2005). Some insect species provide high amino acids concentrations, for example the caterpillars of *Saturniidae*, have a lysine content higher than 100 mg/100 g of CP (Bukkens, 2005).

Most of the experiments published to date have been carried out with broiler chickens fed housefly larvae meal. The results showed that housefly larvae may be added at approximate dietary levels of 25% DM, without any negative effects on weight gain (BWG), feed intake (FI) and feed efficiency (Pretorius, 2011). It suggests that maggot meal may efficiently replace other protein sources, such as soybean meal, fishmeal and groundnut cake. With respect to the metabolizable energy (ME) value of maggot meal, only limited data are available, however, values of 17.9 MJ/kg DM (Zuidhof et al., 2003) for turkey poults and 14.2 MJ/kg DM (Pretorius, 2011) for broilers have been reported (Bovera et al., 2015). Due to variations in fat and "fibre"/chitin content of housefly maggot meal, the ME values vary considerably. Both authors observed a high total tract amino acids digestibility (95%

and 91%, respectively). Feeding maggot meal at the expense of fishmeal at dietary concentrations of 16% to young pullets in the period from 1 to 56 days did not have a negative impact on growth and feed intake of the birds (Engberg et al., unpublished). The feeding of black soldier fly larvae as a substitute for soybean meal resulted in a similar weight gain (BWG) but a lower feed intake (FI) as compared to control indicating an improved feed conversion (FCR) (Makkar et al., 2014).

Mealworms (*T. molitor)* are a pest for feed mills and granaries (Ramos-Elorduy et al., 2002). They are easy to breed and highly nutritious, which is why mealworms are used as feed for pets, as well as exotic species kept in zoos and wildlife parks, including birds, reptiles, mammals, amphibians, and fish. Mealworms are served live, canned dried or lyophilised (Aguilar-Miranda et al., 2002; Hardouin and Mahoux, 2003; Veldkamp et al., 2012). They contain a high amount of crude protein (47–60%) and fat (31–43%). Fresh larvae of *Tenebrio molitor* have a dry matter of 40% and a crude ash content of 1–4.5%. The above mentioned features are reasons for restricting their use in broiler diets (up to 10% of dry matter of whole diet). However, in soybean meal based diets, they may be used without negative effects on feed intake, BWG and FCR (Ramos-Elorduy and Pino, 2002). Additional experiments by Schiavone et al. (2014) show that mealworm meal can be included at maximum dietary concentrations of 25% without causing growth depression. The above mentioned results and high digestibility of nutrients (Table 4) reported by Bovera et al. (2015) show that mealworms are an alternative protein source for soybean meal and fishmeal.

Table 4. Dry matter, organic matter and crude protein ileal digestibility of broilers fed soybean meal or *Tenebrio molitor* larvae meal at 62 d of age (%) (Bovera et al., 2015)

Item	Soybean meal	*Tenebrio molitor*	RMSE	P-value
Dry matter	88.22 a	86.42 b	0.95	0.008
Organic matter	88.69 a	86.86 b	1.01	0.011
Crude protein	87.35 a	80.20 b	0.70	<0.0001

a, b – means within a row with different superscripts differ (P-value<0.01).
RMSE – root mean square error.

Although chickens have been shown to produce chitinase in the proventriculus and hepatocytes (Suzuki et al., 2002), the digestibility of chitin seems to be limited (Hossain and Blair, 2007). Chitin as a polysaccharide may be a substrate for microbial fermentation in the gastrointestinal tract of the chickens and could serve as a substrate for production of chitosan which can have immunomodulatory, antioxidative, antimicrobial, and hypocholesterolemic effects when used as feed additive for poultry (Świątkiewicz et al., 2015). It has been shown that high concentrations (up to 45%) (Muzzarelli, 2013) of chitin present in the cuticular exoskeleton of insects affect the feed intake negatively and reduce protein digestibility (Longvah et al., 2011).

As discussed earlier, another interesting aspect of insects considered for poultry feed is their content of AMPs. These are highly abundant in several species and when used as a feed ingredient, these may reduce the growth of indigenous and potentially pathogenic intestinal bacteria similar to antibiotic growth promoters. In recent studies (Józefiak, unpublished), we used relatively small dietary inclusion levels (up to 0.2%) of low temperature (50°C) dried full fat meals from *Tenebrio molitor, Hermetia illucens* and *Shelfordella lateralis* and observed an improvement of body weight gain in broilers when *Shelfordella lateralis* meal was fed. These results may be explained by the antimicrobial effects on bacterial populations which we have observed in the ileum. In contrast, even low inclusion levels of *Hermetia illucens* full-fat meal impaired broiler performance. However, poultry feeding with fresh insect larvae includes potential risks primarily with regard to feed hygiene, in particular when organic waste products or even manure is used as the medium for larvae growth. In line with this, the "on top" supplementation of living maggots to a balanced diet for young pullets significantly reduced the fearfulness of the birds (Engberg et al., unpublished).

Table 5. Apparent digestibility coefficients of the total tract (CTTAD) of the nutrients, AME and AMEn of insect larval meals for broilers (De Marco et al., 2015)

	Tenebrio molitor	*Hermetia illucens*	SEM	P-value
Dry matter	0.60	0.53	0.02	0.20
Organic matter	0.66	0.66	0.02	0.87
Crude protein	0.60	0.51	0.03	0.23
Ether extract	0.88	0.99	0.02	<0.0001
Gross energy	0.64	0.69	0.02	0.23
AME (MJ/kg DM)	16.86	17.38	0.47	0.59
AMEn (MJ/kg DM)	16.02	16.60	0.46	0.54

Nutritional requirements of insects

The use of insects as "novel" and natural feed materials seems to be an attractive alternative protein source for poultry. However, no nutritional recommendations have been established for this kind of six-legged livestock. There is a huge variation among insects with respect to their nutrient requirements. Until now, the main research efforts have focussed on the insects being members of the orders Diptera (black soldier fly, housefly), Coleoptera (mealworms) and Orthoptera (grasshoppers, locust, crickets and katylids), because these insects were used successfully in many dietary experiments with livestock (Makkar et al., 2014). Their robustness and ability to grow under extreme conditions (low oxygen, no light, high stocking density) as well as their high nutritive value (Table 1, 2 and 3) are of special interest.

For the purposes of large scale insect production, a sequence of tests was conducted to establish the preferential nutritional requirements of feeder insects in order to optimise their growth. Larvae of flies, particularly housefly larvae and black soldier fly larvae, have many features that are highly valued by researchers. Preferences of this order are well known; early in 1928 contemporaneous Chemical Specialties Manufacturers' Association (CSMA) established a referential medium for rearing housefly, which was used at the time for consecutive years. This medium included brans, cellulose or horse manure. One of the researchers (Sawicki, 1964) suggested YMA (yeast-milk agar) as growth medium. Hogsette (1992) offered the enrichment CSMA diet in alfalfa and animal meals (Sawicki, 1964; Hogsette, 1992). At present there are a few applied dietary recommendations for the rearing of housefly maggots. Mealworm breeding and rearing is possible in large scale production using feed materials of a quality that is too low for monogastric animals, e.g. a wide spectrum of industrial and agricultural by-products. Ramos-Elorduy and Pino (2002) observed no significant differences in growth performance of *T. molitor* after 15 days rearing fed with different cereals.

Table 6. Apparent ileal digestibility coefficients (AIDC) of amino acid of the two insect larval meals for broilers (De Marco et al., 2015)

	Tenebrio molitor	*Hermetia illucens*	SEM	P-value
Essential amino acids				
arginine	0.90	0.83	0.03	0.23
histidine	0.85	0.81	0.02	0.44
isoleucine	0.82	0.45	0.05	<0.0001
leucine	0.82	0.76	0.03	0.24
lysine	0.85	0.46	0.05	<0.0001
methionine	0.80	0.42	0.05	<0.0001
phenylalanine	0.91	0.63	0.04	<0.0001
threonine	0.80	0.75	0.03	0.46
valine	0.82	0.62	0.03	<0.0001
mean	0.84	0.64	0.03	<0.0001
Non-essential amino acids				
alanine	0.93	0.86	0.02	0.04
aspartic acid	0.89	0.61	0.04	<0.0001
cysteine	0.84	0.82	0.02	0.52
glycine	0.89	0.67	0.04	<0.0001
glutamic acid	0.88	0.74	0.03	<0.0001
proline	0.84	0.89	0.01	0.06
serine	0.89	0.82	0.03	0.21
tyrosine	0.83	0.43	0.05	<0.0001
mean	0.87	0.73	0.02	<0.0001
Overall mean	0.86	0.68	0.03	<0.0001

Biosecurity and waste management in insect production

Current studies have focussed on the fact that insects from the Diptera order, e.g. the larvae of the housefly and black soldier fly have a great ability to utilize organic waste material (Čičkova et al., 2015; Diener et al., 2011) characterized by high moisture content (60–80%), thus converting it to valuable insect protein. This is particularly attractive for feed and waste management industries. Black soldier fly larvae have been suggested to reduce the accumulation of poultry manure by 50% (Newton et al., 2005). Moreover, the feeding of larvae reduces the amount of available phosphorous in the manure by 61–70% and that of nitrogen by 30–50% (Makkar et al., 2014). A further advantage of these larvae is their ability to reduce bacterial growth in the manure which consequently results in a reduced odour development and the growth suppression of significant pathogens, e.g. *Salmonella* or *E.coli* (Erickson et al., 2004; Liu et al., 2008). As a further co-product, the waste residue of manure can be recycled and used as fertilizer. Even though fly larvae are capable of utilizing animal waste products, e.g. manure and slaughter house offal (Scaglia et al., 2003), it is doubted that these feed items will be approved in the diets for a six-legged livestock and may give rise to biosecurity issues. However, the black soldier fly larvae can also easily grow on plant waste material, e.g. coffee pulp. Recycling of this material constitutes a great problem in countries of Central America. The larvae of the black soldier fly successfully use coffee pulp for growth and at the same time reduce the pH and odour of pulp matter (Lardé, 1990). The organic waste recycling by insects has great perspectives. The most commonly farmed species – *Tenebrio molitor* is also a widely spread pest feeding mainly on grains. Therefore, an optimal diet for this insect should be based on cereal materials (van Broekhoven et al., 2015). Ramos-Elorduy and Pino (2002) and van Broekhoven et al. (2015) demonstrated that *T. molitor* successfully grows and develops on feed including fruit and vegetable waste in various proportions. These experiments show that it is possible to manipulate with the content of nutrients in insect larvae.

The body composition of the insects depends to a large extent on the composition of the nutrients in their diet. This has been shown for insects from the Orthoptera order, in particular the house cricket (*Acheta domestica)*, which is a popular feeder insect for exotic animals held in captivity and is used successfully as laboratory animal. Due to their high protein quality, crickets have also been used in dietary studies with farm animals (Nagakaki et al., 1991; Hatt et al., 2003). These insects need considerable amounts of dietary calcium (up to 6.5%) for normal growth, which makes animal feed containing cricket meal an indirect calcium supplementation. It is possible to successfully increase the amount of calcium even up to 12% in the cricket diet. In this case, the amount of calcium in the body composition of crickets increases significantly, but worsens the results of rearing (Allen, 1989). Hatt et al. (2003) established that it is difficult to change the content of calcium in the body of crickets (even if they get only water for a few weeks), but the Ca:P ratio is unstable and the proper relationship remains only for 48 h. Due to the wide *Acheta domestica* application as laboratory animal, cheaper and more effective rearing methods are being incessantly sought. Currently, the most frequently used materials in cricket diets are cereal products, yeast, grass droughts, vegetables and fruits (Patton, 1967).

The great advantage of insect production is the fact that no additional drinking water has to be applied. As compared to other livestock species, insects utilize water very effectively and in most cases the feed is the main source of water. As the feed supplies both nutrients and water, feed optimization is a very important part of insect production and should be addressed in future research. In our laboratory, work is underway to identify different substrates that are preferred by different insect species. In a choice feeding test (Józefiak et al., unpublished), it was clearly shown that mealworms prefer different roughages rather than wheat middlings or distillers dried grains with solubles (DDGS).

However, we are only in the beginning to understand the special requirements of different insect species used under intensive production conditions, and much work has to be done to optimize the diets to support their optimal growth performance.

Intensive insect production

Considering insects as new six-legged livestock, the prerequisite for any commercial production is knowledge on optimal housing conditions (temperature, humidity and ventilation), and feeding both in terms of feed composition and its structure. Further, it is important to gain knowledge of insect diseases and biosecurity standards for this production form. In contrast to most of the livestock species (excluding fish), insects can be produced in 3D systems, which allows using the buildings very effectively. Moreover, insects can complete some important gaps in the trophic chain, particularly when pre-consumer waste management is taken into consideration as a sustainable insect feed (Figure 2). In this aspect, the most effective are larvae. For instance, it is possible to produce more than 180 kg of live weight of black soldier fly larvae in 42 days from 1 m^2, whereas only 30 kg of adult crickets can be produced on the same area.

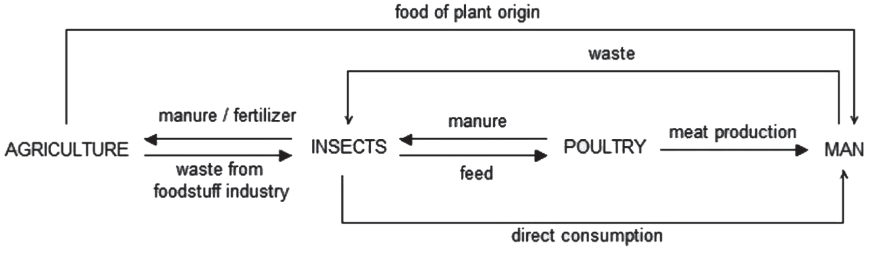

Figure 2. Proposed trophic net among man, poultry, insects and agriculture

The growth rate as well as feed utilization highly depends on temperature, which for most insects is optimal in the range of 27–30°C. Insects are resistant to temperature changes, for instance for mealworms, the optimal rearing temperature is 28°C, but they easily survive 15°C for 48h. However, under high humidity conditions (>70%) they die very quickly.

Another important issue of insect production is farm biosecurity. Depending on the size and activity of invertebrates, very efficient control systems of the buildings have to be applied. For instance, using plastic nets is effective in the production of black soldier flies but not in cockroach or cricket rearing. Therefore, aluminium or copper nets should be used. Finally, intensive insect production should also consider the invasiveness of species which can indirectly affect the natural environment. This is why insect farming should be considered as an "all-in-all-out" system with a separate hatching-brooding area.

Barriers for the inclusion of insect protein in poultry feed

There is no doubt that insect meals from a nutritional perspective are suitable for the feeding of poultry. However, a barrier for the inclusion of insects in feed for livestock is the present EU legislation (Regulation (EC) No. 1069/2009), where insect meals are defined as processed animal protein (PAP). Insects and other invertebrates are classified as Category 3 material (fit but not intended for human food chains). As such, they are suitable as feed for livestock in particular for fish, poultry and pigs. However, despite Regulation (EC) No. 1069/2009, Regulation (EC) No. 999/2001 ("BSE" regulation) prohibits the feeding of farmed animals with PAPs, with the exception of hydrolysed proteins. The feeding of insect meals to aquaculture species is going to be allowed and a re-authorization of these PAPs for pig and poultry feed is expected in the near future. A risk profile related to the production and consumption of insects as food and feed has been recently published by EFSA Scientific committee (2015).

At the moment, a significant obstacle for the use of insects in animal feed is the limited quantity of produced insects, which does not guarantee a constant supply. The prices for insects and insect meals are presently very high, and cannot compete with other protein sources in this respect. To overcome this problem, the most suitable insect species should be identified which has effective protein in terms of production costs on an industrial scale. For mass production, it is necessary to develop automated process technologies for the rearing, harvest and post-harvest procedures, which certainly include the monitoring of product safety and quality (Rumpold and Schlüter, 2013).

The general acceptance of the inclusion of insects in animal feed has been frequently discussed to be a barrier. However, in a recent study from Belgium, cross-sectional data were collected among farmers, agriculture sector stakeholders and citizens (Verbeke et al., 2015). The results of this study indicate a broad acceptance. The perceived benefits such as improved sustainability of livestock production, lower dependence on imported protein sources and lower environmental impact, outweighed the perceived risks, such as microbiological contamination, chemical residues in the food chain and lower consumer acceptance of animal products.

Currently, there are significant knowledge gaps in the field of insect production, particularly in Europe, where insects are not considered a traditional food item (Vantomme et al., 2012; Veldkamp et al., 2012; van Huis et al., 2013). However, it seems that there is nothing stopping us from using insect meals as feed material, so we need to get to work to reduce the costs of insects productions and to remove other limitations in their use in poultry nutrition.

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