


# Nutritional value of insects and ways to manipulate their composition

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## Abstract

This article reports on the nutrients present in insects and factors affecting their variability. Data on protein content and amino acid profiles of a variety of insect species are discussed and their amino acid profiles compared to nutrient requirements of growing broiler chicks, catfish, trout, swine, and human adults and young children. Both *in vitro* and *in vivo* protein digestibility data for a variety of insect species is presented and factors affecting these data are discussed. Furthermore, the fat content and fatty acid profiles of a variety of insect species is reviewed, with special attention on omega-6 and omega-3 fatty acids. Information on carbohydrates, fibre and chitin in insects is shown along with potential effects on nutrient availability. This is followed by a discussion of essential minerals in insects with an emphasis on calcium and phosphorus. Data on insect vitamin content is shown along with a discussion of antinutritional factors such as phytate and thiaminase, which can adversely affect their nutritional value. Dietary effects on insect nutrient composition are reviewed with an emphasis on essential minerals, heavy metals, vitamin E, and carotenoids. Lastly, the effects of processing, including protein extraction and various cooking methods on insect composition are discussed. In summary, this article provides an overview of the nutrient content of insects, and how select nutrients can be altered.

**Keywords:** amino acids, fatty acids, vitamins, minerals, digestibility, nutrient manipulation

## 1. Introduction

Insects are important sources of nutrients for humans and a wide variety of other animal species. Hence studies on insect nutrient composition can be found in disciplines ranging from anthropology to zoology. Comprehensive literature reviews of insect nutrient content have been published (Bukkens, 1997; Finke, 2004; Payne *et al.*, 2016; Raubenheimer and Rothman, 2013; Rumpold and Schluter, 2013a). While the first mention of using insects to feed production animals was in 1919 (Linder, 1919), it was not until the 1960s and 1970s that research started in earnest (Calvert *et al.*, 1969; Hale, 1973; Teotia and Miller, 1973, 1974; Ueckert *et al.*, 1972). The renewed interest in this area of study in the last decade yields numerous papers and comprehensive reviews regarding the safety of insects as food and feed, as well as several overviews of feeding trials for production animals (Gasco *et al.*, 2019; Henry *et al.*, 2015; Makkar *et al.*, 2014; Riddick, 2014; Rumpold and Schluter, 2013b; Sanchez-Muros *et al.*, 2014, 2016). Given the comprehensive nature of these recent reviews, this

article will summarise this compositional data, focus on nutrients that have received little attention and nutrient manipulation. Data on both wild and produced insects is discussed.

## 2. Nutrient content of insects

### Protein and amino acids

The protein content of insects varies between 25 and 75% on a dry matter (DM) basis (Barker *et al.*, 1998; Bukkens, 1997; Cerda *et al.*, 2001; Finke, 2002, 2013, 2015a; Oonincx and Dierenfeld, 2012; Oonincx and Van der Poel, 2011). Proteins are composed of amino acids and the true protein content equals the sum of amino acids. However, protein content is generally estimated by multiplying nitrogen content with a protein factor (Kp) of 6.25. This results in the so-called crude protein content. This factor is underestimated if not all amino acids are quantified, or due to methodological issues (Oonincx *et al.*, 2019b), such as losses of amino acids during hydrolysis. Conversely, the presence of nonprotein

nitrogen from compounds, such as chitin, uric acid, and  $\beta$ -alanine leads to overestimations of true protein content when using this factor (Janssen *et al.*, 2017). An alternative Kp of 4.76 for insects has been suggested based on amino acid data on larvae of yellow mealworms (*Tenebrio molitor*), lesser mealworms (*Alphitobius diaperinus*) and black soldier flies (*Hermetia illucens*) (Janssen *et al.*, 2017). Recalculating the data from 20 insect samples including 13 species and different developmental stages, results in an average Kp of 5.81; range 4.56 to 6.45 (Finke, 2002, 2007, 2013, 2015a,b). This data confirms that a Kp of 6.25 is often a slight overestimate. However, until data for more species and at different life stages are accumulated, retaining a Kp of 6.25 can be beneficial to facilitate comparisons between studies.

The amino acids that make up true protein, are generally grouped as either nutritionally indispensable (essential) amino acids or nutritionally dispensable (non-essential) amino acids. While all amino acids are required, nutritionally indispensable amino acids cannot be synthesised by most animal species and must be provided in the diet. Variation in amino acid patterns between life stages of a species partially depends on whether that species undergoes complete metamorphosis (holometabolous) or incomplete metamorphosis (hemimetabolous) (Finke, 2002; Pieterse and Pretorius, 2014). Comparison of the amino acid pattern (mg amino acid/g crude protein) for the hemimetabolous house cricket (*Acheta domesticus*) suggests that amino acid composition is fairly constant and unaffected by diet or life stage (Table 1). Similarly, black soldier fly prepupae raised on eight different diets had similar amino acid patterns (Spranghers *et al.*, 2017; Wang *et al.*, 2020), as did tobacco hornworm larvae (*Manduca sexta*) fed two different diets (Landry *et al.*, 1986). This suggests that for holometabolous species amino acid patterns are fixed within a specific life stage. Body parts, such as wings, legs or mandibles, have specific physical requirements to function properly; therefore, it is unlikely that their amino acid composition can be altered by diet. Amino acid patterns between different life stages of holometabolous insects would however be expected to differ because larvae and adults are morphologically dissimilar. This was confirmed for yellow mealworms, where the amino acid pattern for larvae differed from the harder bodied adults (Finke, 2002). The adults contain more glycine (52%) and tryptophan (37%) than mealworm larvae, while larvae contain more leucine (29%), phenylalanine (35%) and tyrosine (120%) than adults (Finke, 2002). The amino acid patterns of larvae and pupae meal of the house fly (*Musca domestica*) raised on the same diet also differ (Pieterse and Pretorius, 2014). In this case differences were seen for the amino acids alanine, arginine, aspartic acid, methionine, serine, and tyrosine.

Amino acid patterns are important because they partially determine the suitability of dietary protein sources. This

suitability also depends on the amino acid requirements of the consuming animal. The amino acid with the lowest concentration relative to the requirement of the animal is called the first limiting amino acid. Table 2 shows the amino acid pattern of four commonly raised insect species and compares them to the amino acid requirements of broiler chicks, catfish, trout, swine, and adult humans and young children (NRC, 1994, 2011, 2012; WHO/FAO/UNU, 2007). Methionine and cystine are usually the first limiting amino acids in most insect species when fed to production animals or humans. The exception appears to be house crickets for catfish and swine, where threonine and/or tryptophan are first limiting. These calculations are supported by data from animal feeding trials. Methionine and arginine are the first limiting amino acids for broiler chicks when fed Mormon cricket meal (*Anabrus simplex*) as the sole source of dietary protein in purified diets (Finke *et al.*, 1985), or when house cricket meal is the source of protein in a corn based diet (Nakagaki *et al.*, 1987). Similarly, methionine is the first limiting amino acid in a corn-soy-meat meal diet for growing chickens when dried maggot meal substitutes for meat meal (Bamgbose, 1999). Methionine is also the first limiting amino acid when adult Mormon cricket meal (Finke *et al.*, 1987), yellow mealworm larvae meal (Goulet *et al.*, 1978), or house fly larvae meal are fed to growing rats (Onifade *et al.*, 2001). The high amino acid scores of all four insect species for both children and adults suggests they are a high-quality protein source for humans.

Protein quality is also determined by digestibility and hence amino acid availability. Amino acids from insect meals are readily available when fed to poultry with values equal to, or higher than, those from conventional protein sources, such as soybean meal or fish meal (Table 3). The only exception is black soldier fly larvae meal which has lower amino acid digestibilities especially for the sulphur containing amino acids, methionine and cystine (DeMarco *et al.*, 2015; Schiavone *et al.*, 2017). Larvae (Liland *et al.*, 2017; Schmitt *et al.*, 2019; Tschirner *et al.*, 2015) and prepupae (Spranghers *et al.*, 2017; Wang *et al.*, 2020) of this species have a highly variable mineral content, which is influenced by their diet. As some protein is bound to their mineralised exoskeleton, an increased mineral content might decrease their digestibility. Therefore, amino acid and protein digestibility in poultry might be increased by raising this species on diets with a lower mineral content. Animal performance metrics and insect meal digestibility in shrimp, poultry, ducks, quail, pigs, rabbits, and various species of fish has been elaborately reviewed by Gasco *et al.* (2019).

In a monogastric digestibility model the *in vitro* protein digestibility for dried (conditions unknown) and milled larvae of the yellow mealworm and black soldier fly was only 65.5-68.7% (Marono *et al.*, 2015). Similarly low values were obtained for raw house crickets (65.5%) and

Table 1. Amino acid patterns (mg/g crude protein) of house crickets (*Acheta domesticus*).<sup>1</sup>

Amino acid	Finke (2002) nymphs	Finke (2007) nymphs	Finke (2015b) nymphs	Finke (2002) adults	Finke (2007) adults	Yi <i>et al.</i> (2013) adults	Bosch <i>et al.</i> (2014) adults	Nakagaki <i>et al.</i> (1987)	Poelaert <i>et al.</i> (2018) adults	Köhler <i>et al.</i> (2019) adults
Alanine	89.0	101.1	90.9	87.8	76.9	81.0	83.6	95.0	65.7	82.6
Arginine	61.0	70.9	78.8	61.0	57.3	65.0	56.8	60.0	63.2	64.4
Aspartic acid	70.8	79.4	82.4	83.9	84.9	76.0	76.4	88.0	71.0	103.5
Cystine	8.4	9.1	9.8	8.3	9.8	NR	11.2	NR	12.9	9.1
Glutamic acid	103.9	117.1	114.5	104.9	104.4	110.0	105.9	117.0	98.1	94.8
Glycine	52.6	60.6	53.5	50.7	45.3	51.0	51.3	59.0	45.5	48.1
Histidine	22.1	25.7	22.1	23.4	22.7	21.0	33.9	26.0	31.3	21.7
Isoleucine	42.9	40.6	40.3	45.9	36.4	36.0	40.0	42.0	39.3	38.6
Leucine	95.5	72.6	70.9	100.0	66.7	66.0	66.4	73.0	63.1	75.7
Lysine	53.9	62.3	57.9	53.7	51.1	53.0	58.0	56.0	57.1	55.8
Methionine	13.0	15.4	16.6	14.6	19.6	NR	15.8	15.0	18.7	15.2
Phenylalanine	27.9	32.0	35.6	31.7	30.2	NR	31.8	22.0	31.7	36.0
Proline	55.2	61.1	59.8	56.1	54.2	54.0	54.4	62.0	51.6	50.8
Serine	41.6	42.9	40.4	49.8	52.0	38.0	37.1	49.0	46.2	59.4
Threonine	35.7	38.9	37.6	36.1	31.1	35.0	36.2	35.0	38.5	39.7
Tryptophan	5.2	6.3	8.7	6.3	7.6	9.0	NR	6.0	8.5	9.8
Tyrosine	55.2	62.9	64.8	48.8	44.0	NR	62.1	41.0	47.4	95.7
Valine	49.4	60.0	59.6	52.2	48.4	55.0	57.2	60.0	52.0	64.0

<sup>1</sup> NR = not reported.

yellow mealworms (72.5%) in a porcine model, these were, however, similar to those for beef muscle and chicken breast (Poelaert *et al.*, 2017). Oven cooking or autoclaving further decreased the *in vitro* digestibility of both house crickets and yellow mealworms and also increased neutral detergent fibre, but not acid detergent fibre (Poelaert *et al.*, 2017). Kiiru *et al.* (2020) also reports very low *in vitro* protein digestibility values for raw (29–30.0%) and extruded (38–50%) cricket flours using a static method and quantifying the free amino acids. The latter quantification method is likely to underestimate digestibility as di- and tri-peptides would incorrectly be deemed indigestible. Conversely, higher *in vitro* protein digestibilities (76.4 to 93.3%) were reported for 11 species of freeze-dried insects in a canine model (Bosch *et al.*, 2014, 2016). This included house crickets (91.7%), yellow mealworms (91.3–92.5%), black soldier fly larvae (89.7%) and black soldier fly pupae (77.7%). The *in vitro* protein digestibility of raw yellow mealworms (85.0%) was increased by vacuum cooking (90.5%), boiling (90.1%), oven cooking for 15 and 30 minutes (91.5 and 90.4%, respectively), while frying (87.2%) had no effect (Megido *et al.*, 2018). Kinyuru *et al.* (2010a) also obtained high *in vitro* protein digestibilities (82.3 to 90.5%) for freshly caught termites (*Macrotermes subhylanus*) and grasshoppers (*Ruspolia differens*) that declined slightly after solar drying at approximately 30 °C. Using two different methods, high *in vitro* protein digestibilities were also

reported for yellow mealworm meal (90.2%), and black soldier fly larvae meal (93–94%), when extruded with wheat flour (Azzollini *et al.*, 2018; Ottoboni *et al.*, 2018). The high variability between the aforementioned studies suggest that *in vitro* protein digestibility data should be viewed with caution until it is correlated to *in vivo* studies. Processing and drying methods, as well as the used digestibility model, can have a large impact on data obtained *in vitro*.

Most amino acid availability data of insects pertain to poultry. However, numerous protein digestibility studies have been conducted in rats (Table 4). Typically, the milk protein casein is used as a benchmark in such studies. The digestibility of insect protein was in most cases equal to, or slightly lower, than that for casein. The lower values for protein digestibility in Table 4 for freeze dried yellow mealworms (Goulet *et al.*, 1978), dried termites (*Macrotermes falciger*) (Phelps *et al.*, 1975), and silkworm (*Bombyx mori*) pupae (Rao, 1994) are for apparent digestibility. True digestibility values are higher because they are corrected for endogenous protein/nitrogen losses. True protein digestibility is 7–15% higher than apparent protein digestibility in dried honey bees (*Apis mellifera*) (Ozimek *et al.*, 1985), crickets (Poelaert *et al.*, 2018), yellow mealworms (Jensen *et al.*, 2019; Poelaert *et al.*, 2018) and lesser mealworms (Jensen *et al.*, 2019). Rats fed silkworm pupae consumed 33% less feed than those fed casein, which

**Table 2. Amino acid patterns (mg/g crude protein) of four commonly raised insects and amino acids scores and first limiting amino acid for various species.<sup>1</sup>**

Amino acid	<i>Acheta domesticus</i> adults/nymphs	<i>Tenebrio molitor</i> larvae	<i>Zophobas morio</i> larvae	<i>Hermetia illucens</i> larvae/prepupae
Alanine	87.8	80.2	72.7	62.7
Arginine	65.7	60.0	57.4	52.8
Aspartic acid	79.1	81.0	83.1	88.3
Glutamic acid	109.2	112.1	127.0	103.7
Glycine	52.3	53.1	48.7	55.0
Histidine	22.8	30.2	31.1	32.4
Isoleucine	40.3	46.1	46.9	43.3
Leucine	78.6	84.9	80.4	69.9
Lysine	55.3	55.4	54.6	59.1
Methionine	15.8	13.3	12.2	18.8
Methionine + cystine	24.9	23.3	21.8	24.4
Phenylalanine	31.5	35.2	37.2	41.5
Phenylalanine + tyrosine	87.5	102.6	108.4	112.3
Proline	56.7	68.1	55.9	55.4
Serine	44.1	47.2	44.1	38.3
Threonine	35.7	40.3	39.9	39.0
Tryptophan	7.2	10.5	11.4	15.1
Valine	54.1	62.9	60.5	63.8

Amino acid score/first limiting amino acid:				
Humans				
Children	96/Met+Cys	90/Met+Cys	84/Met+Cys	94/Met+Cys
Adults	113/Met+Cys	106/Met+Cys	99/Met+Cys	111/Met+Cys
Livestock				
Poultry	64/Met+Cys	60/Met+Cys	56/Met+Cys	62/Met+Cys
Catfish	55/Thr+Try	54/Met+Cys	50/Met+Cys	56/Met+Cys
Trout	52/Met+Cys	49/Met+Cys	46/Met+Cys	51/Met+Cys
Swine	59/Try	56/Met+Cys	52/Met+Cys	58/Met+Cys

<sup>1</sup> The amino acid data shown for insects are an average of data from published sources (Finke, 2002, 2007, 2015b; Spranghers *et al.*, 2017; Yi *et al.*, 2013). The insect amino acid scores equal the concentration of their first limiting essential amino acid divided by the concentration of that amino acid in the reference pattern (NRC, (1994, 2011, 2012) for animals and WHO/FAO/UNU (2007) for humans).

**Table 3. Low, high, and mean amino acid digestibilities (%) of various insect meals when fed to poultry.**

Species and life stage	Low	High	Mean	Study
<i>Gryllus testaceus</i> adults	85 (Cys)	96 (Ala)	93	Wang <i>et al.</i> (2005)
<i>Acrida cinerea</i> adults	85 (Cys)	99 (Thr)	94	Wang <i>et al.</i> (2007)
<i>Musca domestica</i> larvae	92 (Ile)	98 (Lys)	95	Hwangbo <i>et al.</i> (2009)
<i>M. domestica</i> larvae	83 (Gly)	96 (Tyr)	91	Pieterse and Pretorius (2014)
<i>M. domestica</i> pupae	86 (Ala)	100 (Ser+Asp)	95	Pieterse and Pretorius (2014)
<i>M. domestica</i> larvae	77 (Gly)	91 (Tyr)	83	Hall <i>et al.</i> (2018)
<i>Tenebrio molitor</i> larvae	80 (Met)	93 (Ala)	86	DeMarco <i>et al.</i> (2015)
<i>Hermetia illucens</i> pupae	42 (Met)	89 (Pro)	68	DeMarco <i>et al.</i> (2015)
<i>H. illucens</i> larvae	44 (Cys)	92 (Ala+Tyr)	77	Schiavone <i>et al.</i> (2017)
<i>H. illucens</i> larvae	45 (Cys)	99 (Ala)	80	Schiavone <i>et al.</i> (2017)

**Table 4. True protein digestibility (%) of various insect species, stages and process methods when fed to rats, compared to benchmark values for protein digestibility of casein.<sup>1</sup>**

Order	Species and life stage	Protein digestibility	Processing method	Casein benchmark	Study		
Coleoptera	<i>Tenebrio molitor</i> larvae meal*	75.1	freeze dried	88.4	Goulet <i>et al.</i> (1978)		
	<i>T. molitor</i> larvae meal + methionine*	78.9	freeze dried	88.4	Goulet <i>et al.</i> (1978)		
	<i>T. molitor</i> larvae meal	91.9	freeze dried	98.8	Poelaert <i>et al.</i> (2018)		
	<i>T. molitor</i> larvae meal	92.0	freeze dried	ND	Jensen <i>et al.</i> (2019)		
	<i>Alphitobius diaperinus</i> larvae meal	93.7	freeze dried	ND	Jensen <i>et al.</i> (2019)		
	<i>A. diaperinus</i> larvae meal	92.5	dried at 120-160 °C	ND	Jensen <i>et al.</i> (2019)		
	<i>A. diaperinus</i> larvae meal	91.5	vacuum dried at 40 °C	ND	Jensen <i>et al.</i> (2019)		
	<i>A. diaperinus</i> larvae meal	94.2	defatted then dried	ND	Jensen <i>et al.</i> (2019)		
	<i>A. diaperinus</i> larvae meal	91.4	extruded then dried at 120 °C	ND	Jensen <i>et al.</i> (2019)		
	<i>A. diaperinus</i> larvae meal	92.4	freeze dried with enzymes added	ND	Jensen <i>et al.</i> (2019)		
	<i>Rhynchophorus phoenicis</i> larvae	91.8	raw	92.6	Ekpo (2011)		
	<i>R. phoenicis</i> larvae	92.6	boiled	92.6	Ekpo (2011)		
	<i>R. phoenicis</i> larvae	92.1	fried	92.6	Ekpo (2011)		
	<i>Oryctes rhinoceros</i> larvae	89.3	raw	92.6	Ekpo (2011)		
	Diptera	<i>Musca domestica</i> larvae meal*	90.6	dried at 65 °C	93.3	Iñiguez-Covarrubias <i>et al.</i> (1994)	
<i>M. domestica</i> larvae meal		89.0-91.0	unknown	89.0-91.0	Kouamé <i>et al.</i> (2011)		
Hymenoptera		dried <i>Apis mellifera</i> adults	71.5	dried at 70 °C	96.8	Ozimek <i>et al.</i> (1985)	
		<i>A. mellifera</i> adult concentrate	94.3	alkaline extraction of bees dried at 70 °C	96.8	Ozimek <i>et al.</i> (1985)	
Isoptera		<i>Macrotermes falciger</i> meal*	45.3-50.7	dewinged, dried and lightly fried	84.2	Phelps <i>et al.</i> (1975)	
		<i>Macrotermes bellicosus</i> adults	90.2	dewinged, raw	92.6	Ekpo (2011)	
		<i>M. bellicosus</i> adults	91.0	dewinged, boiled	92.6	Ekpo (2011)	
		<i>M. bellicosus</i> adults	90.9	dewinged, fried	92.6	Ekpo (2011)	
Lepidoptera		<i>Bombyx mori</i> chrysalid meal	88.9	dried, conditions unknown	99.8	Lin <i>et al.</i> (1983)	
		<i>B. mori</i> chrysalid meal	89.4-89.8	dried, conditions unknown, water extracted	99.8	Lin <i>et al.</i> (1983)	
		<i>B. mori</i> pupae meal *	67.0	unknown	84.0	Rao (1994)	
		<i>Samia ricinii</i> pupae meal	87.0	dried at 60-70 °C and defatted	92.0	Longvah <i>et al.</i> (2011)	
		<i>S. ricinii</i> pupae meal	87.0	dried at 60-70 °C and defatted	92.0	Longvah <i>et al.</i> (2011)	
		<i>Imbrasia belina</i> larvae meal		85.5	gastrointestinal contents removed then dried. drying conditions unknown	98.0	Dreyer and Wehmeyer (1982)
				83.9	gastrointestinal contents removed then dried. drying conditions unknown then soaked in water and canned	98.0	Dreyer and Wehmeyer (1982)
	<i>I. belina</i> larvae		86.0	raw	92.6	Ekpo (2011)	
	<i>I. belina</i> larvae		88.4	boiled	92.6	Ekpo (2011)	
	<i>I. belina</i> larvae		87.8	fried	92.6	Ekpo (2011)	
<i>Clanis bilineata</i> larvae meal	95.8	heads removed and dried at 60 °C	96.1	Xia <i>et al.</i> (2012)			
Orthoptera	<i>Acheta domesticus</i> meal	83.9	freeze dried	98.8	Poelaert <i>et al.</i> (2018)		

<sup>1</sup> \* = apparent protein digestibility; ND = not determined.

would negatively affect apparent protein digestibility (Rao, 1994). Other studies using silkworm meal obtained much higher protein digestibility values. This suggests these low values are not representative. The wide range of protein digestibilities of insects when fed to rats is likely a function of species and life stage, but also how the material was processed prior to use in feeding trials.

Protein digestibility of insects is generally high and influenced by the following factors: (1) if a larger proportion of amino acids is present in cuticular proteins complexed with chitin, or is highly sclerotised protein digestibility is likely decreased (Finke, 2007; Ozimek *et al.*, 1985); (2) removing certain body parts (typically wings, or heads) before further processing and feeding to rats increases

between study variability; (3) processing methods such as drying can decrease protein digestibility depending on time and temperature (Dreyer and Wehmeyer, 1982; Pieterse and Pretorius, 2014).

### Fats and fatty acids

The fat content of insects varies between 10 and 70% on a dry matter basis (Bukkens, 1997; Finke, 2013; Yang *et al.*, 2006). Fat content is typically estimated by an extraction which determines the total weight of all fat-soluble molecules (crude fat). This includes glycerides but also waxes, sterols, fat soluble vitamins and other fat-soluble compounds.

Fat is composed of fatty acids. Two or three fatty acids are coupled to glycerol and form diglycerides and triglycerides, respectively. These fatty acids are divided into saturated, mono-unsaturated, and poly-unsaturated fatty acids based on their degree of saturation. Poly-unsaturated fatty acids are subdivided into omega 3, 6 or 9 poly-unsaturated fatty acids, based on the relative position of the first double bond.

The fatty acid composition of insects depends on the species and life stage as well as environmental factors such as diet, temperature, and light (Finke and Oonincx, 2017). Males of most species have smaller fat reserves than females (Kulma *et al.*, 2019; Lease and Wolf, 2011; Liu *et al.*, 2017; Nestel *et al.*, 2005; Rho and Lee., 2014; Zhou *et al.*, 1995). Commercially produced insects seem to have a higher fat content than those collected from the wild (Finke, 2002, 2013; Lease and Wolf, 2011; Oonincx and Dierenfeld, 2012; Yang *et al.*, 2006). This might be due to decreased energy expenditure in captivity, ready access to diets with a high energy content, or both.

In general, wild caught insects contain relatively high amounts of linoleic acid (18:2 n-6) and linolenic acid (18:3 n-3) (Fast, 1970; Thompson, 1973). Commercially raised insects also contain high levels of linoleic acid but much lower levels of linolenic acid than wild caught insects, because their diet often contains large amounts of grains and grain by-products, which have low levels of linolenic acid (Dreassi *et al.*, 2017; Finke, 2002, 2013; Jones *et al.*, 1972; Oonincx *et al.*, 2015b, 2019a; Paul *et al.*, 2017). Larvae of the black soldier fly have an unusual fatty acid profile rich in lauric acid (C12:0) irrespective of the diet (Finke 2013; Oonincx *et al.*, 2015a; Sprangers *et al.*, 2017; St-Hilaire *et al.*, 2007; Surendra *et al.*, 2016). Like vertebrates, most insects can synthesise saturated and monounsaturated fatty acids (Beenackers *et al.*, 1985; Tietz and Stern, 1969). However, most species are unable to synthesise linoleic acid and linolenic acid, which makes those essential nutrients. There are exceptions such as the American cockroach (*Periplaneta americana*) and the house cricket, which

apparently can synthesise these fatty acids (Borgeson and Blomquist, 1993; Borgeson *et al.*, 1991).

In three insect species with an aquatic larval stage, the concentration of linolenic acid increased, while arachidonic acid (20:4 n-6), and eicosapentaenoic acid (20:5 n-3) decreased in the adults after they emerged from their aquatic habitat (Hanson *et al.*, 1985). This is likely due to differences in the fatty acid content of their diets. Several studies reporting on the fatty acid content of field collected insects find higher levels of eicosapentaenoic acid in aquatic species than in terrestrial species, which rarely contain these fatty acids (Fontaneto *et al.*, 2011; Ghioni *et al.*, 1996; Sushchik *et al.*, 2003, 2013;). In aquatic ecosystems certain microalgae produce eicosapentaenoic acid, which is transferred to higher trophic levels including insects (Gladyshev *et al.*, 2011). Eicosapentaenoic acid is not produced by higher plants in terrestrial ecosystems, therefore herbivorous terrestrial insects are less likely to obtain this fatty acid.

### Carbohydrates

In general, carbohydrates calculated as nitrogen free extract, are present in small amounts in insects (Barker *et al.*, 1998; Finke, 2002, 2013, Oonincx and Dierenfeld, 2011; Pennino *et al.*, 1991). The carbohydrate content of yellow mealworm larvae can vary between one and seven percent (Ramos-Elorduy *et al.*, 2002). However, these differences are likely the result of the food remaining in the gastro-intestinal tract.

### Fibre and chitin

Insects contain significant amounts of fibre as measured by crude fibre, acid detergent fibre, or neutral detergent fibre (Barker *et al.*, 1998; Finke, 2002, 2007, 2013; Lease and Wolf, 2010; Madibela *et al.*, 2007; Marono *et al.*, 2015; Oonincx and Dierenfeld, 2011; Pennino *et al.*, 1991). The components in these fibre fractions are not well known, although in whole insects they include sclerotised proteins and proteins, minerals and other compounds bound to chitin (Finke, 2007; Kramer *et al.*, 1995; Madibela *et al.*, 2007; Marono *et al.*, 2015). Chitin is a N-acetyl- $\beta$ -D-glucosamine polymer, which provides rigidity to the insect's exoskeleton.

The outmost part of insects, the cuticle, is a matrix of proteins, lipids, minerals and other compounds (Kramer *et al.*, 1995). Chitin is only present in the procuticle, the two innermost layers of the cuticle (Moussian, 2010), and therefore makes up a small part of an insect's weight. Quantitative data on the chitin content of whole insects is limited and comparisons between studies are difficult, due to the use of various analytical methods (Cauchie, 2002; Finke, 2007; Henriques *et al.*, 2020; Kaspari, 1991; Lease and Wolf, 2010; Woods *et al.*, 2020). Traditional fibre assays



(crude, neutral detergent or acid detergent) are sometimes used to approximate chitin, even though these methods overestimate true chitin levels (Finke, 2007, 2013; Madibela *et al.*, 2007; Marono *et al.*, 2015). Many animal species, including humans, have chitinases, which can digest chitin (Fujimoto *et al.*, 2002; Lindsay *et al.*, 1984; Paoletti *et al.*, 2007; Strobel *et al.*, 2013; Tabata *et al.*, 2018; Whitaker *et al.*, 2004). To what extent these enzymes are effective *in vivo* is currently unknown.

The predominant compound in the cuticle of most insects is not chitin, but protein (Kramer *et al.*, 1995). ‘Harder bodied’ insects such as adult yellow mealworm beetles contain higher fibre levels than softer bodied insects such as silkworms and house cricket nymphs. This is due to higher levels of amino acids in the acid detergent fibre fraction (Finke, 2007). The amino acid pattern of whole insects differs from the pattern in the acid detergent fibre fraction, and differs between species (Finke, 2007). Particularly valine, histidine, and glycine concentrations are higher in the acid detergent fibre fraction than in whole insects. These amino acids likely contribute to the relative strength, stiffness, elasticity and other physical properties of sclerotised and cuticular proteins. Insects with ‘harder’ cuticles do not necessarily contain more chitin, but contain more sclerotised protein and proteins cross-linked to chitin than softer bodied insects. However, the chitin content of adult beetles does seem higher than in their larvae (Finke, 2007; Kaya *et al.*, 2014; Shin *et al.*, 2019).

## Minerals

Minerals are classified as macro-minerals (calcium, phosphorus, magnesium, sodium, potassium and chloride) and micro or trace minerals (iron, zinc, copper, manganese, iodine and selenium). This classification is based on the amount needed to meet dietary requirements. For most species, requirements for macro-minerals are measured in g per kg and micro-mineral requirements in mg per kg.

Insects generally contain low levels of calcium because they lack a mineralised skeleton. Calcium levels are typically less than 0.3% DM although higher levels have been reported for stoneflies (Plecoptera) and some other species (Barker *et al.*, 1998; Finke, 2002, 2013; Oonincx and Dierenfeld, 2012; Oonincx and Van der Poel, 2011; Punzo, 2003; Studier and Sevick, 1992). Higher levels of calcium occasionally reported for feeder crickets are likely due to dietary calcium remaining in the gut (Barker *et al.*, 1998; Finke, 2002; Frye and Calvert, 1989; Hatt *et al.*, 2003; Oonincx and Dierenfeld, 2011; Oonincx and Van der Poel, 2011; Punzo, 2003). While the exoskeleton of most insects is primarily composed of protein and chitin, black soldier fly larvae (Finke, 2013; Spranghers *et al.*, 2017), and face fly larvae (*Musca autumnalis*) (Dashefsky *et al.*, 1976; Koo *et al.*, 1980; Roseland *et al.*, 1985), have a mineralised exoskeleton in

which calcium and other minerals are incorporated into the cuticle. Therefore, they can contain high levels of calcium.

Most insects contain more phosphorus than calcium, except for species with a mineralised exoskeleton (i.e. face fly and black soldier fly larvae). Significant amounts of phosphorus are present in plants as phytate and are hence unavailable for digestion. In contrast, phosphorus in insects is likely to be bioavailable as was reported when face fly pupa were fed to poultry (Dashefsky *et al.*, 1976).

Magnesium levels in insects likely meet the dietary requirements of most species (Barker *et al.*, 1998; Finke, 2002, 2013; Oonincx and Dierenfeld, 2012; Studier and Sevick, 1992). Black soldier fly larvae contain 3 to 10 times more magnesium than most other insects (Finke, 2013; Spranghers *et al.*, 2017). This is likely the result of their mineralised exoskeleton in which minerals such as calcium and magnesium form a complex with chitin (Diener *et al.*, 2015).

Only a few studies report the sodium and potassium content of insects (Finke, 2002, 2013; Oonincx and Dierenfeld, 2011; Oonincx and Van der Poel, 2011; Reichle *et al.*, 1969; Studier and Sevick, 1992). Insects typically contain more potassium than sodium whereas chloride levels are intermediate. Most insect species likely contain adequate amounts of these three minerals to meet the dietary requirements of most animal species.

Most insects contain sufficiently high levels of the trace minerals iron, zinc, copper, manganese and selenium, which would meet the dietary requirements for most animals (Barker *et al.*, 1998; Finke, 2002, 2013; Oonincx and Dierenfeld, 2012; Oonincx and Van der Poel, 2011; Punzo, 2003; Studier and Sevick, 1992). However, studies suggest possible species-specific differences for certain trace minerals. For example, the manganese concentrations in the alates of five termite species (2,710-5,150 mg/kg DM) were extremely high compared to mopane worms (*Gonimbrasia belina*; 39 mg/kg DM), house crickets (38 mg/kg DM), yellow mealworms (5 mg/kg DM), or migratory locusts (10 mg/kg DM) determined in the same study (Verspoor *et al.*, 2020). However, other termite species from other regions, for instance *Nasutitermes* spp., from Venezuela, reportedly have lower manganese concentrations (32-115 mg/kg DM) (Oyarzun *et al.*, 1996). Caste differences were apparent in the latter study; alates had a far lower manganese concentration than workers (37 vs 115 mg/kg DM).

Adult fruit flies (*Drosophila melanogaster*) (Barker *et al.*, 1998; Oonincx and Dierenfeld, 2012), and house flies (Finke, 2013), contain relatively high levels of iron (125 to 454 mg/kg DM) compared to most other insect species. Iron and zinc concentrations in insects seem positively correlated

(Spearman's  $\rho=0.592$ ), that is higher iron concentrations often coincide with higher zinc concentrations (Mwangi *et al.*, 2018). The contents of the gastrointestinal tract can be a significant percentage of the total weight of an insect (4-7% of the live weight) (Finke, 2003); and therefore diet also directly influences the (trace) mineral content of fully fed insects.

Mineral availability can be inhibited by so-called anti-nutritional factors such as phytate, oxalate, and tannins. For example, caterpillars of the Pallid emperor moth (*Cirina forda*) contain both phytic acid (10 mg/kg) and oxalate (40 mg/kg), but no tannins (Omotoso, 2006). Tannins (10.4 mg/kg), phytic acid (13.5 mg/kg) and oxalates (0.8 mg/kg) were found in larvae of the African palm weevil (*Rhynchophorus phoenicis*) (Ekpo, 2011). The source of these anti-nutritional factors is likely from the food present in the gastrointestinal tract. Evidence of accumulation of these substances in insects is lacking. The concentrations found seem to be relatively low; in seeds and cereal grains concentrations up to 7% DM have been found (Zhou and Erdman, 1995).

## Vitamins and carotenoids

### Vitamin A

Vitamin A is a group of compounds composed of retinoids and carotenoids. Like most vertebrates, insects obtain retinoids via the cleavage of various carotenoids (Von Lintig, 2012). However, carotenoid cleavage in vertebrates primarily takes place in the intestine, and the resulting retinoids are stored in the liver (Olson, 1989), whereas insects convert carotenoids to retinoids only in the compound eye (Von Lintig, 2012). In fruit flies and honey bees (*Apis mellifera*) retinoids are only found in the compound eye while other parts of the adult insect do not contain retinoids nor do fruit fly larvae (Giovannucci and Stephenson, 1999; Goldsmith and Warner, 1964). This explains why holometabolous adults contain very low levels of vitamin A/retinoids (Barker *et al.*, 1998; Finke, 2002, 2013; Oonincx and Dierenfeld, 2012; Oonincx and Van der Poel, 2011; Pennino *et al.*, 1991), and holometabolous larvae, which lack compound eyes, do not contain preformed retinoids (retinal or 3-hydroxyretinal).

In many animal species certain carotenoids can be converted to vitamin A/retinal (Bender, 2002; Levi *et al.*, 2012; McComb, 2010; Olson, 1989). High levels of carotenoids, including those that can be converted to retinal, are found in various wild insect species (Arnold *et al.*, 2010; Cerda *et al.*, 2001; Eeva *et al.*, 2010; Isaksson and Andersson, 2007; Newbrey *et al.*, 2013; Seki *et al.*, 1998; Ssepuuya *et al.*, 2017) whereas commercially produced insects contain far lower quantities (Finke, 2002, 2013, 2015a; Oonincx and Van der Poel, 2011). This difference is likely a result of dietary carotenoid intake as was shown

for both silkworm larvae (Chieco *et al.*, 2019) and fruit flies (Giovannucci and Stephenson, 1999).

### Vitamin D

For a long time, insects were considered to contain low levels of vitamin D (typically <400 IU/kg DM) (Finke, 2002, 2013, Oonincx *et al.*, 2010). However, data from three wild caught species indicated a high variability in its concentrations with values ranging from below the detection limit (100 IU vitamin D3/kg DM) up to 1,288 IU vitamin D3/kg DM (Finke, 2015b). More recently it was discovered that certain insects, like vertebrate animals, can synthesise vitamin D3 *de novo* when exposed to UV-B (Oonincx *et al.*, 2018). This capacity varies greatly between species; no evidence of *de novo* synthesis is found in black soldier fly larvae, but yellow mealworm larvae can reach over 6,000 IU/kg DM (Oonincx *et al.*, 2018).

### Vitamin E

Vitamin E content of insects, including house crickets, yellow mealworm larvae and black soldier fly larvae, varies. Values for house crickets range from 8 to 195 IU vitamin E/kg DM, yellow mealworm larvae range from <22 to 116 IU vitamin E/kg DM and black soldier fly larvae range from 10 to 235 IU vitamin E/kg DM (Finke, 2002; Pennino *et al.*, 1991). This variation is likely due to dietary differences resulting in different amounts being incorporated in the insect's tissues and dietary vitamin E in the insect's gastrointestinal tract. While variable between species, the vitamin E content of most commercially raised insects is below 37 IU/kg DM (Barker *et al.*, 1998; Finke, 2002, 2013; Oonincx and Dierenfeld, 2011). Wild insects appear to contain higher levels of vitamin E than commercially raised insects, which are often provided with diets containing low levels of vitamin E (Pennino *et al.*, 1991; Punzo, 2003).

### B-vitamins

Several studies report on the B-vitamin content of commercially raised insects (Bawa *et al.*, 2020; Finke, 2002, 2013, 2015b; Jones *et al.*, 1972). However, the B-vitamin content of unprocessed wild-caught insects is limited (Finke, 2015b; Igwe *et al.*, 2011; Kinyuru *et al.*, 2010a,b). Some information on B-vitamin content of wild-caught insects consumed by humans is available (Banjo *et al.*, 2006; Dreyer and Wehmeyer, 1982; Igwe *et al.*, 2011; Kinyuru *et al.*, 2010a; Kodondi *et al.*, 1987; Santos Oliveira *et al.*, 1976; Teffo *et al.*, 2007). These, however, are usually dried, boiled, fried, or roasted and specific body parts are often removed. During this processing some B-vitamins can be destroyed by exposure to heat, light or oxygen. Furthermore, differences in sample preparation and analytical methods (microbiological versus chemical techniques) complicate direct comparisons.



Despite these issues some general observation regarding B-vitamins in insects can be made. Most studies show unprocessed insects are a very good source of riboflavin (vitamin B2), niacin, pantothenic acid, pyridoxine (vitamin B6), biotin, folic acid, and cyanocobalamin (vitamin B12). Low levels for several of these vitamins occasionally reported in the literature are likely due to losses during processing and storage (Kinyuru *et al.*, 2010a).

One B-vitamin that appears to be low in many species of insects is thiamine (vitamin B1). Many species of commercially raised or wild caught insects including house crickets, adult yellow mealworms, superworms (*Zophobas morio*), butterworms, (*Chilecomadia moorei*) Turkistan roaches (*Blatta lateralis*), pallid wing grasshoppers (*Trimerotropis pallidipennis*), and rhinoceros beetles (*Oxygryllus ruginasus*) contain low levels of thiamine (<3.0 mg/kg DM) although others (black soldier fly larvae, adult house flies, silkworms, yellow mealworm larvae, waxworms (*Galleria mellonella*), and white-lined sphinx moths (*Hyles lineata*) contain much higher levels (5.0 to 45.0 mg thiamine/kg DM) (Bawa *et al.*, 2020; Finke, 2002, 2013, 2015b). This variation is similar to that observed for 14 insect species collected in Nigeria (0.3 to 32.4 mg thiamine/kg dry mater) (Banjo *et al.*, 2006) and three species from Angola (1.3 to 36.7 mg thiamine/kg DM) (Santos Oliveira *et al.*, 1976). Dried and smoked Attacidae caterpillars (*Nudaurelia melanops*, *Imbrasia truncata*, *Imbrasia epimethea*) from Zaire contain low thiamine levels (1.5 to 2.7 mg/kg dry product) (Kodondi *et al.*, 1987) and unprocessed longhorn grasshoppers from East Africa contained no detectable thiamine (Kinyuru *et al.*, 2010b). Because thiamine is relatively unstable and most of the values reported are for insects processed (i.e. body parts removed and dried, smoked or fried), it is unclear how representative these values are for unprocessed insects. That said, the range of values for thiamine in processed insects is similar to that observed for raw insects.

Two species of edible insects (domesticated silkworm larvae and African silkworm pupae (*Anaphe* spp.) contain the enzyme thiaminase (Nishimune *et al.*, 2000). Unless inactivated during processing, thiaminase destroys thiamine. Thiamine deficiency due to the consumption of *Anaphe* pupae is suggested as a cause of a seasonal ataxia in local populations in Nigeria (Adamolekun, 1993; Adamolekun *et al.*, 1997). Most enzymes are inactivated by heat, but some thiaminase activity remains after brief exposure of the extract of *Anaphe* pupae to 100 °C, suggesting it is heat tolerant. This thiaminase had an optimal activity at 70 °C and only after 15 minutes exposure to 100 °C was it largely deactivated (Nishimune *et al.*, 2000). The gut of the variegated grasshopper (*Zonocerus variegatus*) also contains a thiaminase (Ehigie *et al.*, 2013). This, however, has its optimal activity at a lower temperature (50 °C) indicating it is a different enzyme. It

is currently unknown how widespread thiaminases are in insects.

### Choline

Choline is a component of both lecithin and the neurotransmitter acetylcholine. It also plays an important role in one-carbon metabolism and as such can spare the need for dietary methionine (Pesti *et al.*, 1979). The data on choline in insects, albeit limited, indicate insects contain high levels of choline (1,570 to 7,258 mg/kg DM) (Finke, 2002, 2013, 2015a,b; Fogang *et al.*, 2017; Noland and Baumann, 1949). Because methionine is typically the first-limiting amino acid when fed to most species, choline content is important when using insects as food or feed.

### Taurine

Most animal species can synthesise taurine from its precursor methionine. For some species, such as cats and foxes, this amino sulfonic acid is a required nutrient. In general insect larvae contain little, if any, taurine (Bodnaryk, 1981; Finke, 2002, 2013, 2015a; Massie *et al.*, 1989; Ramsey and Houston, 2003). Larvae of both the bertha armyworm (*Mamestra configurata*) and fruit flies contain little (<200 µg/g fresh weight), or undetectable taurine but levels increase in pupae and peak in adults (Bodnaryk, 1981; Massie *et al.*, 1989) with values ranging from 500 to 1,100 µg/g fresh weight, likely because insect flight muscles contain high concentrations of taurine (Whitton *et al.*, 1987). Adult house crickets (Finke, 2002, 2015a), adult pallid wing grasshoppers (Finke, 2015b), adult fruit flies (Massie *et al.*, 1989), house flies (Finke, 2013), and adult moths (Bodnaryk, 1981; Finke, 2015b) are rich sources of taurine, while three species of adult beetles (*T. molitor*, *O. ruginasus* and an unreported species) contain only low levels (Finke, 2002, 2015b; Ramsey and Houston, 2003).

### Sterols

Unlike most animals, insects cannot synthesise the characteristic ring structure of sterols and hence, require a dietary source (Jing and Behmer, 2020). The sterol form therefore depends on the diet and the insect species. Phytosterols such as stigmasterol, sitosterol, and campesterol, or zoosterols such as cholesterol and 7-dehydrocholesterol, are enzymatically converted for their required function. Typically they form structural components of phospholipid bilayers in cell membranes, but are also used as precursors for vitamin D or moulting hormone. Total sterol content and the variation seems a function of species, diet, life stage and season (Connor *et al.*, 2006; Košťál *et al.*, 2013; Liland *et al.*, 2017). While insects contain a variety of sterols often β-sitosterol, cholesterol, or 7-dehydrocholesterol are the most abundant (Cerdeira *et al.*, 2001; Cheseto *et al.* 2015; Connor *et al.*, 2006; Jing and

Behmer, 2020; Košťál *et al.*, 2013; Liland *et al.*, 2017; Mlček *et al.*, 2019; Sabolová *et al.*, 2016; Svoboda *et al.*, 1995). High dietary levels of sterols, such as cholesterol, can increase the risk of atherosclerosis in humans and some species of monkeys (Hopkins, 1992; Rudel *et al.*, 1998).

### 3. Life stage effects on fat content

Fat stores are usually highest in the final larval or nymphal stage (Fast, 1970). This pattern depends to some extent on whether the species is holometabolous or hemimetabolous. Larvae of holometabolous species have a higher fat content than adults (Lease and Wolf, 2011; Punzo, 2003). For instance, the fat content of the larvae of fruit flies (Church and Robertson 1966), house flies (Pearincott, 1960), black soldier flies (Liu *et al.*, 2017) and yellow mealworms (Finkel, 1948) increases as they develop but drops significantly in the adult because fat is used as an energy source during pupation. A similar decrease is observed in insects undergoing diapause or hibernation (Ali and Ewiess, 1977; Downer and Matthews, 1976).

Similar to holometabolous insects, nymphs of hemimetabolous insects slowly increase their fat content as they develop as was shown for house crickets (Hutchins and Martin, 1968; Lipsitz and McFarlane, 1971). However, unlike holometabolous insects, the fat contents of newly emerged hemimetabolous adults are similar to late stage nymphs and then slowly decline (Lipsitz and McFarlane, 1971).

### 4. Dietary effects on insect nutrient composition

Diet can affect the composition of fats/fatty acids, vitamins, carotenoids, and minerals in insects. The amount and the fatty acid composition of insect fat is highly variable and affected by both life stage and diet. This subsequently affects the levels of other nutrients, most notably protein and moisture. An increase in fat content, with stable amounts of moisture and protein, dilutes the concentrations of the latter two as was shown for house fly larvae (Pearincott, 1960).

#### Fat

The extent to which dietary changes in crude fat content and fatty composition of insects are possible depends strongly on the species. For instance, the fat content of yellow mealworms fed four different diets ranged from 23 to 29% DM, while Argentinean cockroaches (*Blaptica dubia*) fed these diets ranged from 16 to 40% DM (Oonincx *et al.*, 2015a). Similarly, the fat content of house crickets fed five different diets ranged from 9 to 44% (Bawa *et al.*, 2020). The fat content of velvet bean caterpillars (*Anticarsia gemmatilis*) is 40 to 65% higher on an artificial diet compared to leaves from three plants (Cookman *et al.*, 1984). This increase in fat content carried through to the adult stage; moths from

larvae fed the artificial diet contained 71 to 105% more fat than those fed plant leaves (Cookman *et al.*, 1984). Similarly, higher fat contents were found in dried insect meals from larvae of the tobacco hornworm (21 vs 17% dry DM) and the fall armyworm (*Spodoptera frugiperda*) (21 vs 12% DM) fed artificial diets, compared to those fed leaves or grasses (Landry *et al.*, 1986).

Besides the variability in total fat content, large differences in fatty acid composition of insects when fed different diets are apparent, which is similar to most vertebrates. Numerous studies indicate that the fatty acid composition in both larval and adult insects partially reflects the fatty acid composition of the diet (Cookman *et al.*, 1984; Madariaga *et al.*, 1971; Schaefer, 1968; Spranghers *et al.*, 2017; St-Hilaire *et al.*, 2007; Van Broekhoven *et al.*, 2015). However, due to selective accumulation, catabolism and/or fatty acid synthesis these fatty acid compositions are not identical (Cookman *et al.*, 1984; Madariaga *et al.*, 1971; Oonincx *et al.*, 2015a; Schaefer, 1968).

The omega-3 fatty acid content of house fly larvae (Hussein *et al.*, 2017), black soldier fly larvae (Erbland *et al.*, 2020; Oonincx *et al.*, 2019a; St-Hilaire *et al.*, 2007), Jamaican field crickets (Komprda *et al.*, 2013; Starčević *et al.*, 2017), house crickets (Finke 2015b; Oonincx *et al.*, 2019a), yellow mealworms (Fasel *et al.*, 2017; Finke 2015b; Oonincx *et al.*, 2019a), lesser mealworms (Oonincx *et al.*, 2019a), superworms, and waxworms (Finke, 2015b; Komprda *et al.*, 2013), are increased by providing a diet enriched with these fatty acids. Black soldier fly larvae fed diets containing fish oil or fish by-products accumulate both eicosapentaenoic acid and docosahexaenoic acid (Erbland *et al.*, 2020; St-Hilaire *et al.*, 2007). In contrast house crickets, superworms and waxworms fed diets containing fish oil accumulate eicosapentaenoic acid but not docosahexaenoic acid, even though both were present in the diets (Finke, 2015b). To which extent this is due to species, diet, or experimental setup requires further studies. When, and for how long these fatty acids should be provided will partially depend on the further use of the insect. Addition at the final developmental stage (i.e. gut-loading) might be suitable if whole insects, including the gastrointestinal contents, are used.

#### Vitamins

There are little data regarding the effect of diet on the vitamin content in insects. Supplementing the diets of house crickets, yellow mealworms, superworms, and waxworms with high levels of vitamin E during growth, resulted in high levels in the insects (116-440 IU/kg DM) (Finke, 2015a). Because the insects were fasted prior to analysis, these elevated levels are mainly due to incorporation of vitamin E into the tissue of the insect. This is similar to swine, where elevated levels of dietary vitamin E increase

the vitamin E concentration of various tissues (Asghar *et al.*, 1991). Carotenoid content can also be increased via the insect's diet. Feeding a diet containing  $\beta$ -carotene to species that use retinal as their chromophore such as house crickets, yellow mealworm larvae, and superworm larvae increases  $\beta$ -carotene concentration in their tissues (Finke, 2015a). Providing a diet containing  $\beta$ -carotene to species that use 3-hydroxyretinal as their chromophore such as Diptera and Lepidoptera typically does not increase  $\beta$ -carotene but elevates zeaxanthin levels as was shown for blow flies (*Calliphora*) (Vogt and Kirschfeld, 1984), fruit flies (Giovannucci and Stephenson, 1999), and waxworm larvae (Finke, 2015b). However, pupae from two silkworm strains did contain some  $\beta$ -carotene when the larvae were fed diets containing  $\beta$ -carotene (Chieco *et al.*, 2019). Fruit fly larvae accumulate carotenoids and convert these to retinoids when they form compound eyes during pupation (Seki *et al.*, 1998; Von Lintig, 2012).

### Minerals and heavy metals

To some extent mineral composition can be altered by diet, depending on the mineral and the species of insect. Across three different studies the calcium content of black soldier fly prepupae fed nine different diets ranged from 1 to 66 g/kg DM (Proc *et al.*, 2020; Spranghers *et al.*, 2017; Wang *et al.*, 2020). Iron, zinc, and manganese also varied while phosphorus and potassium were similar across dietary treatments. The mineral content of black soldier fly larvae fed sixteen different diets also varied greatly (8 to 83 g/kg DM) although in this case part of the effects are likely a result of the diet remaining in the gastrointestinal tract of the larvae (Liland *et al.*, 2017; Schmitt *et al.*, 2019; Tschirner *et al.*, 2015). The mineralised exoskeleton of black soldier fly larvae might be the reason for these extreme variations. Zinc also accumulates in black soldier fly larvae, prepupae and adults when incorporated in the larval diet (Diener *et al.*, 2015). Zinc accumulates in larval and prepupal exuvia, further indicating that the variability in mineral concentrations is due to the mineralised exoskeleton of this species. In housefly pupae, reared on diet spiked with zinc chloride, the zinc concentration increased by approximately 10% of the dietary concentration, which might indicate effects from gut loading, rather than selective accumulation and storage (Maryanski *et al.*, 2002). Like zinc, the heavy metals copper, cadmium and lead accumulate in black soldier fly larvae (Diener *et al.*, 2015; Purschke *et al.*, 2017; Van der Fels-Klerx *et al.*, 2016; Wu *et al.*, 2020). A measure for the efficiency of accumulation is the bio accumulation factor (BAF). It is calculated by dividing the concentration in the insect, by the concentration in the diet. Hence, a BAF above 1 indicates selective accumulation and a BAF below 1 indicates selective excretion. The aforementioned studies indicate that the BAF for arsenic, mercury, chrome, and nickel are below 1 in the black soldier fly. However, contrasting data has been reported by Schmitt *et al.* (2019)

confirming accumulation of cadmium and mercury and selective excretion for lead and copper. The accumulation of cadmium seems to occur in several fly species, including houseflies, fruit flies, several midge species, and the flesh fly *Boettcherisca peregrina* (Charlton *et al.*, 2015; Gao *et al.*, 2017; Kazimírová and Ortel, 2000; Maroni and Watson, 1985; Maryanski *et al.*, 2002; Postma *et al.*, 1996; Purschke *et al.*, 2017; Timmermans and Walker, 1989; Wu *et al.*, 2006).

In contrast to these Diptera, larvae of the yellow mealworm larvae accumulate arsenic, but not copper, zinc, lead or cadmium (Van der Fels-Klerx *et al.*, 2016; Vijver *et al.*, 2003). The concentrations of lead and cadmium in this species can, however, increase partially due to the presence of these elements in the gastro-intestinal tract (Vijver *et al.*, 2003). This was for instance shown for the predatory beetle (*Notiophilus biguttatus*), which efficiently excreted retained cadmium when it was removed from the diet (Jansen *et al.*, 1991). Similarly, the predatory carabid beetle (*Poecilus cupreus*) had increased cadmium levels (approximately 10% of the dietary concentration), and zinc (less than 10% of the dietary concentration) when provided with spiked diets (Maryanski *et al.*, 2002). However, when provided with a cadmium free diet, concentrations fell rapidly (Kramarz, 1999). The cadmium concentration in the sago grub was below 10% of the dietary concentration (Köhler *et al.*, 2020) and no evidence of cadmium or zinc accumulation was found in the beetle *Neochetina eichhorniae* (Jamil and Hussain, 1992).

Several studies on heavy metals in Orthopterans, either taken from contaminated sites or experimentally exposed, have been conducted. Higher levels in the environment lead to higher levels of lead, cadmium, and mercury in four species of grasshoppers (Devkota and Schmidt, 2000). The BAF for cadmium was above 1, whereas it was below 1 for lead. Interestingly, the BAF for mercury was below 1 for three of the species, but >2 for the fourth. A higher BAF for cadmium than for lead was also reported for the Oriental longheaded grasshopper (*Acrida chinensis*) and migratory locusts (Zhang *et al.*, 2012). Both cadmium and copper concentrations increased in migratory locusts with increased dietary levels (Crawford *et al.*, 1996). The same was also found in the common field grasshopper (*Chorthippus brunneus*), and it was shown that most of the cadmium and copper (85% for both) accumulated in the integument (Hunter *et al.*, 1987). This might explain why lower concentrations are often found in later instar nymphs and adults which have a lower surface to volume ratio.

Jamaican field crickets (*Gryllus assimilis*) also accumulate dietary cadmium (Bednarska *et al.*, 2015). However, their zinc levels remain constant at different dietary levels indicating that zinc concentrations are more tightly regulated. Tight regulation of heavy metals that are of

nutritional relevance, such as iron, zinc, and copper, seems common over a variety of insect species. This is plausible, as a variety of selective transporters are present to regulate their concentrations within the insect (Mwangi *et al.*, 2018).

Tobacco cutworms (*Spodoptera litura*) also concentrate copper and zinc from their diet in amounts that seem to fit physiological levels (7 and 70 mg/kg fresh weight, respectively) (Zhuang *et al.*, 2009). Lead, and especially cadmium, are largely excreted by this lepidopteran. A noteworthy ecological study on the Bogong moth (*Agrotis infusa*) indicates that they store arsenic and can effectively transport this pollutant during migration (Green, 2008).

Based on the information above, it seems that accumulation and excretion patterns show similarities at the order level. However, these patterns differ between life stages (Lindqvist, 1992; Timmermans and Walker, 1989), and species-specific exceptions likely exist. Therefore, if a new species is to be commercially produced, controlled studies on accumulation and excretion patterns are advisable. Variation in dietary intake levels will to some extent affect insect concentrations. For species collected from the field, seasonal differences in host plants and host plant concentrations will affect insect concentrations (Ortiz *et al.*, 2015; Zhang *et al.*, 2009). Furthermore, insects from contaminated areas can have elevated concentrations due to contaminated diets, but might also take up these minerals directly from the environment.

## 5. Environmental effects on insect composition

Environmental factors such as temperature, light, and humidity are known to affect growth and development (Akman Gündüz and Gülel 2002; Ali and Ewiess, 1977; Ali *et al.*, 2011; Han *et al.*, 2008; Roe *et al.*, 1980, 1985). Some effects on chemical composition are also known to occur. For instance, bean beetles (*Acanthoscelides obtectus*) raised at 30 °C contain more protein and the same amount of fat as their counterparts raised at 20 °C (Sönmez and Gülel, 2008). An increase in rearing temperature from 20 to 27 °C in two-spotted field crickets (*Gryllus bimaculatus*) decreases their protein concentration and increases the concentration of fat (Hoffmann, 1973). Their fatty acid profile also changes with higher temperatures leading to a higher degree of saturation. This coincides with an increased fresh weight per individual, possibly indicating an increased synthesis of saturated fatty acids at 27 °C. Similarly, females of the mosquito, *Culex tarsalis*, contain more unsaturated fatty acids (C16:1, C18:1 and C18:2) when raised at 22 °C, compared to counterparts raised at 30 °C which contain more short chain fatty acids (Harwood and Takata, 1965). This accumulation of unsaturated fatty acids is more pronounced when this species is raised under a short photoperiod, which would increase cold survival. Indeed, photoperiodic effects on insect nutrient content

are likely indirect, acting through other processes such as preparing for diapause.

Besides photoperiod, the emission spectrum of the light source can influence composition. If UV-B is emitted, this will lead to vitamin D3 synthesis in certain insects such as the yellow mealworm (Oonincx *et al.*, 2018). The vitamin D3 concentration increases over time with prolonged exposure until it reaches a plateau. A higher plateau is reached with a higher UV-B intensity. A higher abundance of short wavelengths within the UV-B spectrum is also likely to lead to a higher vitamin D3 content in insects, as was shown in the vertebrate *Pogona vitticeps* (Diehl *et al.*, 2018).

## 6. Processing effects on insect nutrient composition

Although specific information on the effects of various processing methods on the nutrient content and availability of insect products is limited, it is likely similar to other human foods or animal feeds (Ssepuyua *et al.*, 2017). Heat and processing effects on protein/amino acid availability, effects of traditional cooking methods on trace mineral, fatty acid and fat content and processing effects on B-vitamins in insect and insect products have been reported.

Processing using high pressures and temperatures decreases amino acid availability and protein quality (Batterham *et al.*, 1986). As such, the protein quality of insect meals is likely decreased by processing conditions, especially drying temperatures. The lower average amino acid availability of house fly larvae meal (91%), compared to house fly pupae meal (95%), might partially be due to longer drying times (Pieterse and Pretorius, 2014). Likewise, dried Mopane caterpillars exposed to high temperatures during canning have a slightly reduced apparent protein digestibility compared to those that are not canned (83.9 and 85.8%, respectively) (Dreyer and Wehmeyer, 1982). In contrast, no effect of processing (either boiling or frying) on protein digestibility or biological value was seen for four species of insects when fed to rats (Ekpo, 2011). Similarly, Jensen *et al.* (2019) report that neither protein digestibility, nor the biological value of lesser mealworm larvae is altered by freeze drying, vacuum drying at low (40 °C), or higher (120-160 °C) temperatures, industrial drying, defatting, extrusion, or by adding a blend of enzymes to the diet when fed to rats.

In addition to processing insects into meals for animal feed, protein extraction from raw insects for use in human food has also been evaluated (Lin *et al.*, 1983; Ozimek *et al.*, 1985; Yi *et al.*, 2013). Dried and ground silkworm chrysalid protein was compared to an aqueous extract of ground silkworm chrysalid protein with and without pretreatment with hydrogen peroxide (Lin *et al.*, 1983). Amino acid patterns were similar for the three treatments;

however, sodium hydroxide treatment prior to extraction decreased cysteine levels. Whereas protein digestibility was similar between treatments, net protein utilisation was lower for the protein extract pre-treated with sodium hydroxide, probably due to cysteine destruction.

While some processing methods negatively affect protein/ amino acid availability, some can improve protein quality. Ozimek *et al.* (1985) evaluated the effect on amino acid pattern and availability and the net protein value of an alkaline extraction of dried adult honeybees fed to rats. This extraction increased protein quality of the concentrate compared to dried honeybees by elevating the relative abundance (amino acid/protein) of leucine (54%) and methionine (59%). Amino acid availability was also improved by extraction (90%), compared to dried honeybees (65.4%), and was similar to casein (92.2%). The improved amino acid availability may partially be due to the removal of cuticular proteins complexed with chitin; the alkaline extraction decreased chitin content from 11.1% DM to below the detection limit. The improved amino acid profile and increased amino acid availability in the extract improved net protein utilisation from 41.5 to 60.6% compared to dried adult honeybees.

The nutrient content of insects prepared for human consumption may be altered by traditional cooking methods in two ways. Depending on the type of cookware used, iron, zinc, copper, and nickel can leach from the cookware and into the insect during preparation. The high values for copper, iron and zinc reported for some insects prepared using traditional methods may be partially due to the cookware used (Payne *et al.*, 2015; Santos Oliveira *et al.*, 1976). Additionally, many insects consumed by humans are either broiled, roasted, or fried prior to consumption. Broiled or roasted insects contain less fat than raw insects as fat is lost during cooking. A comparison of traditional processing methods using four insect species (black soldier fly prepupae, house crickets, longhorn grasshoppers and the Egyptian cotton leafworm (*Spodoptera littoralis*)) showed decreases in fat content (0.8-51%) and consequently increases of the crude protein (1.2-22%) content (Nyangena *et al.*, 2020). Fat content was the highest in the raw product and decreased in the following order: toasting, boiling, oven drying and solar-drying.

For fried insects, the fat content increases as the fat from the cooking oil is absorbed by the insect (Santos Oliveira *et al.*, 1976). This also changes the fatty acid profiles, because this material contains both the fat naturally present in the insect and the fat in the cooking oil (Santos Oliveira *et al.*, 1976).

As previously mentioned, some B-vitamins are relatively unstable when exposed to heat, light, or oxygen. The vitamin content of insects processed for human consumption is lower than raw unprocessed insects, possibly due to

losses during cooking and drying. Kinyuru *et al.*, (2010a) evaluated the effect of toasting (frying at approximately 150 °C for 5 minutes) and drying (solar drying at 30 °C) on the niacin, riboflavin, pyridoxine, and folic acid content of Mendi termites and longhorn grasshoppers. Toasting decreased the folic acid content of these species by 37 and 43%, respectively, and riboflavin by 23 and 34%, respectively. In contrast there was little loss of pyridoxine and niacin. Drying of termites and grasshoppers also decreased both folic acid by 47 to 66%, and riboflavin by 29 to 46%. There was little loss of pyridoxine during drying and a significant loss of niacin for termites (26%), but not for grasshoppers. The reason for these differences is unclear. Of note is that thiamine (vitamin B1) which is known to be unstable during heating was not measured in this study.

Dreyer and Wehmeyer (1982) showed that canning reduces thiamine (84%), riboflavin (20%) and niacin (42%) levels in milled Mopane caterpillars, most likely because of the heat used to sterilise the cans. In contrast to the loss of B-vitamins,  $\beta$ -carotene was unaffected by the canning process.

## 7. Conclusions

Insects have a high protein content and their amino acid profiles are suitable for production animals and humans alike. This protein is highly digestible; hence, insects are generally a good source of dietary protein. In most cases methionine is the first limiting amino acid when insects are used as food or feed. Fat is the next most prominent nutrient in insects. Large variations in fat content and fatty acid composition occur due to species, life stage, diet and gender.

Carbohydrates are virtually absent in the insect body but fibre is present in significant amounts. This fibre consists primarily of chitin and sclerotised proteins, which are components of the insect exoskeleton. Whereas calcium levels in insects are lower than in animals with an endoskeleton, most other minerals are present in adequate concentrations to meet nutrient requirements of animals and humans. Mineral concentrations can be especially high in certain fly larvae. Vitamin A levels in insects are low, however carotenoids can be present in relatively high concentrations, depending on the diet. Vitamin D content is highly variable and largely depends on the availability of UV-B radiation during insect development. Also vitamin E concentrations are highly variable and depend on dietary levels. Most B-vitamins are present in adequate concentrations in most insect species. The taurine content of insects is highly variable and depends on both species and life stage.

Several factors such as species and life stage influence the composition of insect species. Diet can strongly affect the

concentration of most nutrients, although some, especially amino acids and minerals of nutritional relevance, tend to be less flexible. Heavy metals can accumulate in insects. However, accumulation patterns vary between species and orders. Insect processing can affect their nutritional value in many ways, but the most common are destruction of vitamins and denaturation of proteins due to heat. Although large variations in insect composition are known, in general they are to be considered a prime source of nutrients.

## Conflict of interest

The authors declare no conflict of interest.

## References

- Adamolekun, B., 1993. *Anaphe venata* entomophagy and seasonal ataxic syndrome in southwest Nigeria. *Lancet* 341: 629.
- Adamolekun, B., McCandless, D.W. and Butterworth, R.F., 1997. Epidemic of seasonal ataxia in Nigeria following ingestion of the African silkworm *Anaphe venata*: role of thiamine deficiency? *Metabolic Brain Disease* 12: 251-258.
- Akman Gündüz, N.E. and Gülel, A., 2002. Effect of temperature on development, sexual maturation time, food consumption and body weight of *Schistocerca gregaria* Forsk. (Orthoptera: Acrididae). *Türk Zooloji Dergisi* 26: 223-227.
- Ali, M. and Ewiess, M.A., 1977. Photoperiodic and temperature effects on rate of development and diapause in the green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae). *Zeitschrift Fur Angewandte Entomologie* 84: 256-264.
- Ali, M.F., Mashaly, A.M.A., Mohammed, A.A. and El-Magd Mahmoud Mohammed A., 2011. Effect of temperature and humidity on the biology of *Attagenus fasciatus* (Thunberg) (Coleoptera: Dermestidae). *Journal of Stored Product Research* 47: 25-31. <https://doi.org/10.1016/j.jspr.2010.07.002>
- Arnold, K.E., Ramsay, S.L., Henderson, L. and Larcombe, S., 2010. Seasonal variation in diet quality: antioxidants, invertebrates and blue tits *Cyanistes caeruleus*. *Biological Journal of the Linnean Society* 99: 708-717.
- Asgar, A., Gray, J.I., Miller, E.R., Ku, P.K., Booren, A.M. and Buckley, D.J., 1991. Influence of supranutritional vitamin E supplementation in the feed on swine growth performance and deposition in different tissues. *Journal of the Science of Food and Agriculture* 57: 19-29.
- Azzollini, D., Derossi, A., Fogliano, V., Lakemond, C.M.M. and Severini, C., 2018. Effects of formulation and process conditions on microstructure, texture and digestibility of extruded insect-riched snacks. *Innovative Food Science and Emerging Technologies* 45: 344-353.
- Bamgbose, A.M., 1999. Utilization of maggot-meal in cockerel diets. *Indian Journal of Animal Sciences* 69: 1056-1058.
- Banjo, A.D., Lawal, O.A. and Songonuga, E.A., 2006. The nutritional value of fourteen species of edible insects in southwestern Nigeria. *African Journal of Biotechnology* 5: 298-301.
- Barker, D., Fitzpatrick, M.P. and Dierenfeld, E.S., 1998. Nutrient composition of selected whole invertebrates. *Zoo Biology* 17: 123-134.
- Batterham, E.S., Darnell, R.E., Herbert, L.S. and Major, E.J., 1986. Effect of pressure and temperature on the availability of lysine in meat and bone meal as determined by slope-ratio assays with growing pigs, rats and chicks and by chemical techniques. *British Journal of Nutrition* 55: 441-453.
- Bawa, M., Songsempong, S., Kaewtapee, C. and Chanput, W., 2020. Effect of diet on the growth performance, feed conversion, and nutrient content of the house cricket. *Journal of Insect Science* 20: 1-10.
- Bednarska, A.J., Opyd, M.O., Zurawicz, E. and Laskowski, R., 2015. Regulation of body metal concentrations: Toxicokinetics of cadmium and zinc in crickets. *Ecotoxicology and Environmental Safety* 119: 9-14.
- Beenackers, M.T.A., Van der Horst, D.J. and Van Marrewijk, W.J.A., 1985. Insect lipids and lipoproteins, and their role in physiological processes. *Progress in Lipid Research* 24: 19-67.
- Bender, D.A., 2002. Introduction to nutrition and metabolism. CRC Press, London, UK, 450 pp.
- Bodnaryk, R.P., 1981. The biosynthesis function, and fate of taurine during the metamorphosis of the Noctuid moth *Mamestra configurata* Wlk. *Insect Biochemistry* 11: 199-205.
- Borgeson, C.E. and Blomquist, G.J., 1993. Subcellular location of the  $\Delta^{12}$  desaturase rules out bacteriocyte contribution to linoleate biosynthesis in the house cricket and the American cockroach. *Insect Biochemistry and Molecular Biology* 23: 297-302.
- Borgeson, C.E., Kurtti, T.J., Munderloh, U.G. and Blomquist, G.J., 1991. Insect tissues, not microorganisms, produce linoleic-acid in the house cricket and the American cockroach. *Experientia* 47: 238-241.
- Bosch, G., Vervoort, J.J.M. and Hendriks, W.H., 2016. *In vitro* digestibility and fermentability of selected insects for dog foods. *Animal Feed Science and Technology* 221: 174-184.
- Bosch, G., Zhang, S., Oonincx, D.G.A.B. and Hendriks, W.H., 2014. Protein quality of insects as potential ingredients for dog and cat foods. *Journal of Nutritional Science* 3: e29.
- Bukkens, S.G.F., 1997. The nutritional value of edible insects. *Ecology of Food and Nutrition* 36: 287-319.
- Calvert, C.C., Martin, R.D. and Morgan, N.O., 1969. House fly pupae as food for poultry. *Journal of Economic Entomology* 62: 938-939.
- Cauchie, H.M., 2002. Chitin production by arthropods in the hydrosphere. *Hydrobiologia* 470: 63-96.
- Cerda, H., Martinez, R., Briceno, N., Pizzoferrato, L., Manzi, P., Tommaseo Ponzetta, M., Marin, O. and Paoletti, M.G., 2001. Palm worm: (*Rhynchophorus palmarum*) traditional food in Amazonas, Venezuela – nutritional composition, small scale production and tourist palatability. *Ecology of Food and Nutrition* 40: 13-32.
- Charlton, A., Dickinson, M., Wakefield, M., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M. and Devic, E., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. *Journal of Insects as Food and Feed* 1: 7-16. <https://doi.org/10.3920/JIFF2014.0020>
- Cheseto, X., Kuate, S.P., Tchouassi, D.P., Ndung'u, M., Teal, P.E. and Torto, B., 2015. Potential of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) as an unconventional source of dietary and therapeutic sterols. *PLoS ONE* 10: e0127171.



- Chieco, C., Morrone, L., Bertazza, G., Cappellozza, S., Saviane, A., Gai, F., Di Virgilio, N. and Rossi, F., 2019. The effect of strain and rearing medium on the chemical composition, fatty acid profile and carotenoid content in silkworm (*Bombyx mori*) pupae. *Animals* 9: 103.
- Church, R.B. and Robertson, F.W., 1966. A biochemical study of the growth of *Drosophila melanogaster*. *Journal of Experimental Zoology* 162: 337-351.
- Connor, W.E., Wang, Y., Green, M. and Lin, D.S., 2006. Effects of diet and metamorphosis upon the sterol composition of the butterfly *Morpho peleides*. *Journal of Lipid Research* 47: 1444-1448.
- Cookman, J.E., Angelo, M.J., Slansky Jr, F. and Nation, J.L., 1984. Lipid content and fatty acid composition of larvae and adults of the velvetbean caterpillar, *Anticarsia gemmatilis*, as affected by larval diet. *Journal of Insect Physiology* 30: 523-527.
- Crawford, L.A., Lepp, N.W. and Hodkinson, I.D., 1996. Accumulation and egestion of dietary copper and cadmium by the grasshopper *Locusta migratoria* R and F (Orthoptera: Acrididae). *Environmental Pollution* 92: 241-246. [https://doi.org/10.1016/0269-7491\(96\)00004-8](https://doi.org/10.1016/0269-7491(96)00004-8)
- Dashefsky, H.S., Anderson, D.L., Tobin, E.N. and Peters, T.M., 1976. Face fly pupae: a potential feed supplement for poultry. *Environmental Entomology* 5: 680-682.
- DeMarco, M., Martinez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L. and Schiavone, A., 2015. Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. *Animal Feed Science and Technology* 209: 211-218.
- Devkota, B. and Schmidt, G.H., 2000. Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. *Agriculture, Ecosystems and Environment* 78: 85-91.
- Diehl, J.J.E., Baines, F.M., Heijboer, A.C., Van Leeuwen, J.P., Kik, M., Hendriks, W.H. and Oonincx, D.G.A.B., 2018. A comparison of UV b compact lamps in enabling cutaneous vitamin D synthesis in growing bearded dragons. *Journal of Animal Physiology and Animal Nutrition* 102: 308-316.
- Diener, S., Zurbrugg, C. and Tockner, K., 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. *Journal of Insects and Food and Feed* 1: 261-270. <https://doi.org/10.3920/JIFF2015.0030>
- Downer, R.G.H. and Matthews, J.R., 1976. Patterns of lipid distribution and utilisation in insects. *American Zoologist* 16: 733-745.
- Dreassi, E., Cito, A., Zanfini, A., Materozzi, L., Botta, M. and Francardi, V., 2017. Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Lipids* 52: 285-294.
- Dreyer, J.J. and Wehmeyer, A.S., 1982. On the nutritive value of mopanie worms. *South African Journal of Science* 78: 33-35.
- Eeva, T., Helle, S. and Salminen, J.P., 2010. Carotenoid composition of invertebrates consumed by two insectivorous bird species. *Journal Chemical Ecology* 36: 608-613.
- Ehigie, L.O., Emuebie, O. and Ehigie, F.A., 2013. Biochemical properties of thiaminase, a toxic enzyme in the gut of grasshoppers (*Zonocerus variegatus* Linn). *Cameroon Journal of Experimental Biology* 9: 9-16.
- Ekpo, K.E. 2011. Effect of processing on the protein quality of four popular insects consumed in Southern Nigeria. *Archives of Applied Science Research* 3: 307-326.
- Erbland, P., Alyokhin, A., Perkins, L.B. and Peterson, M., 2020. Dose-dependent retention of omega-3 fatty acids by black soldier fly larvae (Diptera: Stratiomyidae). *Journal of Economic Entomology* 113: 1221-1226. <https://doi.org/10.1093/jeet/toaa045>
- Fasel, N.J., Mène-Safrané, L., Ruczynski, I., Komar, E. and Christe, P. 2017. Diet induced modifications of fatty acid composition in mealworm larvae (*Tenebrio molitor*). *Journal of Food Research* 6: 5. <https://doi.org/10.5539/jfr.v6n5p22>
- Fast, P.G., 1970. Insect lipids. *Progress in the Chemistry of Fats and other Lipids* 11: 181-242.
- Finke M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology* 21: 269-285.
- Finke, M.D., 2003. Gut loading to enhance the nutrient content of insects as food for reptiles: a mathematical approach. *Zoo Biology* 22: 147-162.
- Finke, M.D. 2004. The nutrient content of insects. In: Capinara, J.L. (ed.) *Encyclopedia of entomology*. Vol 2. Kluwer Academic Publishers, Dordrecht, the Netherlands, p. 1562-1575.
- Finke, M.D., 2007. Estimate of chitin in raw whole insects. *Zoo Biology* 26: 105-115.
- Finke, M.D., 2013. Complete nutrient content of four species of feeder insects. *Zoo Biology* 32: 27-36.
- Finke, M.D., 2015a. Complete nutrient content of three species of wild caught insects, pallid-winged grasshopper, rhinoceros beetles and white-lined sphinx moth. *Journal of Insects as Food and Feed* 1: 281-292. <https://doi.org/10.3920/JIFF2015.0033>
- Finke, M.D., 2015b. Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology* 34: 554-564.
- Finke, M.D. and Oonincx, D.G.A.B., 2017. Nutrient content of insects. In: Van Huis, A. and Tomberlin, J.K. (eds) *Insects as food and feed: from production to consumption*. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 290-317.
- Finke, M.D., DeFoliart, G.R. and Benevenga, N.J., 1987. Use of a four-parameter logistic model to evaluate the protein quality of mixtures of Mormon cricket meal and corn gluten meal in rats. *Journal of Nutrition* 117: 1740-1750.
- Finke, M.D., Sunde, M.L. and DeFoliart, G.R., 1985. An evaluation of the protein quality of Mormon crickets (*Anabrus simplex* Haldeman) when used as a high protein feedstuff for poultry. *Poultry Science* 64: 708-712
- Finkel, A.J., 1948. The lipid composition of *Tenebrio molitor* larvae. *Physiological Zoology* 21: 111-133.
- Fogang, A.R., Kansci, G., Viau, M., Hafnaoui, N., Meynier, A., Demmano, G. and Genot, C., 2017. Lipid and amino acids profiles support the potential of *Rhynchophorus phoenicis* larvae for human nutrition. *Journal of Food Composition and Analysis* 60: 64-73.
- Fontaneto, D., Tommaseo-Ponzetta, M., Galli, C., Risé, P., Glew, R.H. and Paoletti, M.G., 2011. Differences in fatty acid composition between aquatic and terrestrial insects used as food in human nutrition. *Ecology of Food and Nutrition* 50: 351-367.

- Frye, F.L. and Calvert, C.C., 1989. Preliminary information on the nutritional content of mulberry silk moth (*Bombyx mori*) larvae. *Journal of Zoo and Wildlife Medicine* 20: 73-75.
- Fujimoto, W., Suzuki, M., Kimura, K. and Iwanaga, T., 2002. Cellular expression of the gut chitinase in the stomach of frogs *Xenopus laevis* and *Rana catesbeiana*. *Biomedical Research-Tokyo* 23: 91-99.
- Gao, Q., Wang, X., Wang, W., Lei, C. and Zhu, F., 2017. Influences of chromium and cadmium on the development of black soldier fly larvae. *Environmental Science and Pollution Research* 24: 8637-8644.
- Gasco, L., Biasato, I., Dabbou, S., Schiavone, A. and Gai, F., 2019. Animals fed insect-based diets: State-of-the-art on digestibility, performance and product quality. *Animals* 9: 170. <https://doi.org/10.3390/ani9040170>
- Ghioni, C., Bell, J.G. and Sargent, J.R., 1996. Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. *Comparative Biochemistry and Physiology B* 114: 161-170.
- Giovannucci, D.R. and Stephenson, R.S., 1999. Identification and distribution of dietary precursors of the *Drosophila* visual pigment chromophore: analysis of carotenoids in wild type and *ninaD* mutants by HPLC. *Vision Research* 39: 219-229.
- Gladyshev, M.I., Kharitonov, A.Y., Popova, O.N., Sushchik, N.N., Makhutova, O.N. and Kalacheva, G.S., 2011. Quantitative estimation of dragonfly role in transfer of essential polyunsaturated fatty acids from aquatic to terrestrial ecosystems. *Doklady Biochemistry and Biophysics* 438: 141-143.
- Goldsmith, T.H. and Warner, L.T., 1964. Vitamin A in the vision of insects. *Journal of General Physiology* 47: 433-441.
- Goulet, G., Mullier, P., Sinave, P. and Brisson, G.J., 1978. Nutritional evaluation of dried *Tenebrio molitor* larvae in the rat. *Nutrition Reports International* 18: 11-15.
- Green, K., 2008. Migratory bogong moths (*Agrotis infusa*) transport arsenic and concentrate it to lethal effect by estivating gregariously in alpine regions of the Snowy Mountains of Australia. *Arctic, Antarctic, and Alpine Research* 40: 74-80.
- Hale, O.M., 1973. Dried *Hermetia illucens* (Stratiomyidae) as a feed additive for poultry. *Journal of the Georgia Entomological Society* 8: 16-20.
- Hall, H.N., Masey O'Neill H.V., Scholey D., Burton E., Dickinson M. and Fitches E.C., 2018. Amino acid digestibility of larval meal (*Musca domestica*) for broiler chickens. *Poultry Science* 97: 1290-1297.
- Han, R.D., Parajulee, M., Zhong, H. and Feng, G., 2008. Effects of environmental humidity on the survival and development of pine caterpillars, *Dendrolimus tabulaeformis* (Lepidoptera: Lasiocampidae). *Insect Science* 15: 147-152.
- Hanson, B.J., Cummins, K.W., Cargill, A.S. and Lowry, R.R., 1985. Lipid content, fatty acid composition, and the effect of diet on fats of aquatic insects. *Comparative Biochemistry and Physiology B* 80: 257-276.
- Harwood, R.F. and Takata, N., 1965. Effect of photoperiod and temperature on fatty acid composition of the mosquito *Culex tarsalis*. *Journal of Insect Physiology* 11: 711-716
- Hatt, J.M., Hung, E. and Wanner, M., 2003. The influence of diet on the body composition of the house cricket (*Acheta domesticus*) and consequences for their use in zoo animal nutrition. *Der Zoologische Garten* 73: 238-244.
- Henriques, B.S., Garcia, E.S., Azambuja, P. and Genta, F.A., 2020. Determination of chitin content in insects: an alternative method based on calcofluor staining. *Frontiers in Physiology* 11: 117. <https://doi.org/10.3389/fphys.2020.00117>
- Henry, M., Gascob, L., Piccoloc, G. and Fountoulakia, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. *Animal Feed Science and Technology* 203: 1-22.
- Hoffmann, K.H., 1973. Effects of temperature on chemical composition of crickets (*Gryllus*, orthopt.) (In German). *Oecologia* 13: 147-175.
- Hopkins, P.N., 1992. Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review. *American Journal of Clinical Nutrition* 55: 1060-1070.
- Hunter, B.A., Hunter, L.M., Johnson, M.S. and Thompson, D.J., 1987. Dynamics of metal accumulation in the grasshopper *Chorthippus brunneus* in contaminated grasslands. *Archives of Environmental Contamination and Toxicology* 16: 711-716.
- Hussein, M., Pillai, V.V., Goddard, J.M., Park, H.G., Kothapalli, K.S., Ross, D.A., Ketterings, Q.M., Brenna, J.T., Milstein, M.B., Marquis, H., Johnson, P.A., Nyrop, J.P. and Selvaraj, V., 2017. Sustainable production of housefly (*Musca domestica*) larvae as a protein-rich feed ingredient by utilizing cattle manure. *PLoS ONE* 12: e0171708.
- Hutchins, R.F.N. and Martin, M.M., 1968. The lipids of the common house cricket, *Acheta domesticus* L. I. Lipid classes and fatty acid distribution. *Lipids* 3: 247-249.
- Hwangbo, J., Hong, E.C., Jang, A., Kang, H.K., Oh, J.S., Kim, B.W. and Park, B.S., 2009. Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. *Journal of Environmental Biology* 30: 609-614.
- Igwe, C.U., Ujowundu, C.O., Nwaogu, L.A. and Okwu, G.N., 2011. Chemical analysis of an edible African termite, *Macrotermes nigeriensis*, a potential antidote to food security problem. *Biochemistry and Analytical Biochemistry* 1: 1-4.
- Iñiguez-Covarrubias, G., De Franco-Gómez, M.J. and Del R Andrade-Maldonado, G., 1994. Biodegradation of swine waste by house-fly larvae and evaluation of their protein quality in rats. *Journal of Applied Animal Research* 5: 65-74.
- Isaksson, C. and Andersson, S., 2007. Carotenoid diet and nestling provisioning in urban and rural great tits *Parus major*. *Journal of Avian Biology* 38: 564-572.
- Jamil, K. and Hussain, S., 1992. Biotransfer of metals to the insect *Neochetina eichhornae* via aquatic plants. *Archives of Environmental Contamination and Toxicology* 22: 459-463.
- Janssen, M., Bruins, A., De Vries, T. and Van Straalen, N., 1991. Comparison of cadmium kinetics in four soil arthropod species. *Archives of Environmental Contamination and Toxicology* 20: 305-312.
- Janssen, R.H., Vincken, J.P., Van den Broek, L.A.M., Fogliano, V. and Lakemond, C.M.M., 2017. Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *Journal of Agricultural and Food Chemistry* 65: 2275-2278.
- Jensen, L.D., Miklos, R., Dalsgaard, T.K., Heckmann, L.H. and Nørgaard, J.V., 2019. Nutritional evaluation of common (*Tenebrio molitor*) and lesser (*Alphitobius diaperinus*) mealworms in rats and processing effect on the lesser mealworm. *Journal of Insects as Food and Feed* 5: 257-266. <https://doi.org/10.3920/JIFF2018.0048>

- Jing, X. and Behmer, S.T., 2020. Insect sterol nutrition: physiological mechanisms, ecology, and applications. *Annual Review of Entomology* 65: 251-271.
- Jones, L.D., Cooper, R.W. and Harding, R.S., 1972. Composition of mealworm *Tenebrio molitor* larvae. *The Journal of Zoo Animal Medicine* 3: 34-41.
- Kaspari, M., 1991. Prey preparation as a way that grasshopper sparrows (*Ammodramus savannarum*) increase the nutrient composition of their prey. *Behavioral Ecology* 2: 234-241.
- Kaya, M., Baran, T., Erdoğan, S., Menteş, A., Aşan Özusağlam, M. and Çakmak, Y.S., 2014. Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (*Leptinotarsa decemlineata*). *Materials Science and Engineering: C* 45: 72-81.
- Kazimírová, M. and Ortel, J., 2000. Metal accumulation by *Ceratitis capitata* (Diptera) and transfer to the parasitic wasp *Coptera occidentalis* (Hymenoptera). *Environmental Toxicology and Chemistry* 19: 1822-1829.
- Kiiru, S.M., Kinyuru, J.N., Kiage, B.N. and Marel, A.K. 2020. Partial substitution of soy protein isolates with cricket flour during extrusion affects firmness and *in vitro* protein digestibility. *Journal of Insects as Food and Feed* 6: 169-177. <https://doi.org/10.3920/JIFF2019.0024>
- Kinyuru, J.N., Kenji, G.M., Muhoho, S.N. and Ayieko, M. 2010b. Nutritional potential of longhorn grasshopper (*Ruspolia differens*) consumed in Siaya district, Kenya. *Journal of Agriculture, Science and Technology* 12: 32-46.
- Kinyuru, J.N., Kenji, G.M., Njoroge, S.M. and Ayieko, M. 2010a. Effect of processing methods on the *in vitro* protein digestibility and vitamin content of edible winged termite (*Macrotermes subhyllanus*) and grasshopper (*Ruspolia differens*). *Food Bioprocess Technology* 3: 778-782.
- Kodondi, K.K., Leclercq, M. and Gaudin-Harding, F., 1987. Vitamin estimations of three edible species of Attacidae caterpillars from Zaire. *International Journal of Vitamin and Nutrition Research* 57: 333-334.
- Köhler, R., Irias-Mata, A., Ramandey, E., Purwestri, R. and Biesalski, H.K., 2020. Nutrient composition of the Indonesian sago grub (*Rhynchophorus bilineatus*). *International Journal of Tropical Insect Science* 40: 677-686. <https://doi.org/10.1007/s42690-020-00120-z>
- Köhler, R., Kariuki, L., Lambert, C. and Biesalski, H.K., 2019. Protein, amino acid and mineral composition of some edible insects from Thailand. *Journal of Asia-Pacific Entomology* 22: 372-378.
- Komprda, T., Zornikova, G., Rozikova, V., Borkovcova, M. and Przywarova, A., 2013. The effect of dietary *Salvia hispanica* seed on the content of n-3 long-chain polyunsaturated fatty acids in tissues of selected animal species, including edible insects. *Journal of Food Composition and Analysis* 32: 36-43.
- Koo, S.I., Currin, T.A., Johnson, M.G., King, E.W. and Turk, D.E., 1980. The nutritional value and microbial content of dried face fly pupae (*Musca autumnalis* (De Geer)) when fed to chicks. *Poultry Science* 59: 2514-2518.
- Košťál, V., Urban, T., Rimnáčová, L., Berková, P., and Simek, P., 2013. Seasonal changes in minor membrane phospholipid classes, sterols and tocopherols in overwintering insect, *Pyrrhocoris apterus*. *Journal of Insect Physiology* 59: 934-41.
- Kouamé, B., Marcel, G., Brou André, K., Alassane, M., Koffi Gabouet, K. and Coulibally Séraphi, K., 2011. Détermination du taux optimal de farine d'asticots séchés dans le régime du rat en croissance. *Journal of Animal and Plant Sciences* 12: 1553-1559.
- Kramarz, P., 1999. Dynamics of accumulation and decontamination of cadmium and zinc in carnivorous invertebrates. 1. The ground beetle, *Poecilus cupreus* L. *Bulletin of Environmental Contamination and Toxicology* 63: 531-537.
- Kramer, K.J., Hopkins, T.L. and Schaefer, J., 1995. Applications of solids NMR to the analysis of insect sclerotized structures. *Insect Biochemistry and Molecular Biology* 25: 1067-1080.
- Kulma, M., Kouřimská, L., Plachý, V., Božik, M., Adámková, A. and Vrabec, V., 2019. Effect of sex on the nutritional value of house cricket, *Acheta domestica* L. *Food Chemistry* 272: 267-272.
- Landry, S., DeFoliart, G.R. and Sunde, M.L., 1986. Larval protein quality of six species of Lepidoptera (Saturniidae, Sphingidae, Noctuidae). *Journal of Economic Entomology* 79: 600-604.
- Lease, H.M. and Wolf, B.O., 2010. Exoskeletal chitin scales isometrically with body size in terrestrial insects. *Journal of Morphology* 271: 759-768.
- Lease, H.M. and Wolf, B.O., 2011. Lipid content of terrestrial arthropods in relation to body size, phylogeny, ontogeny and sex. *Physiological Entomology* 36: 29-38.
- Levi, L., Ziv, T., Admon, A., Levavi-Sivan, B. and Lubzens, E., 2012. Insight into molecular pathways of retinal metabolism, associated with vitellogenesis in zebrafish. *American Journal of Physiology – Endocrinology and Metabolism* 302: 626-644.
- Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbø, R., Bente, E. Torstensen, B.E., and Lock, E.J., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLoS ONE* 12: e0183188.
- Lin, S., Njaa, L.R., Eggum, B.O. and Shen, H., 1983. Chemical and biological evaluation of silk worm chrysalid protein. *Journal of the Science of Food and Agriculture* 34: 896-900.
- Linder, P., 1919. Extraction of fat from small animals. *Zoological Technology and Biology* 7: 213-220.
- Lindqvist, L., 1992. Accumulation of cadmium, copper, and zinc in five species of phytophagous insects. *Environmental entomology* 21: 160-163.
- Lindsay, G.J.H., Walton, M.J., Adron, J.W., Fletcher, T.C., Cho, C.Y. and Cowey, C.B., 1984. The growth of rainbow trout (*Salmo gairdneri*) given diets containing chitin and its relationship to chitinolytic enzymes and chitin digestibility. *Aquaculture* 37: 315-334.
- Lipsitz, E.Y. and McFarlane, J.E., 1971. Analysis of lipid during the life cycle of the house cricket, *Acheta domesticus*. *Insect Biochemistry* 1: 446-460.
- Liu, X., Chen, X., Wang, H., Yang, Q., Ur Rehman, K., Li, W., Cai, M., Li, Q., Mazza, L., Zhang, J., Yu, Z., and Zheng, L., 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS ONE* 12: e0182601.
- Longvah, T., Mangthya, K. and Ramulu, P., 2011. Nutrient composition and protein quality evaluation of eri silkworm (*Samia racinii*) prepupae and pupae. *Food Chemistry* 128: 400-403.



- Madariaga, M.A., Mata, F., Municio, A.M. and Ribera, A. 1971. Effect of the lipid composition of the larval diet on the fatty acid composition during development of *Ceratitis capitata*. *Insect Biochemistry* 2: 249-256.
- Madibela, O.R., Seitiso, T.K., Thema, T.F. and Letso, M., 2007. Effect of traditional processing methods on chemical composition and *in vitro* true dry matter digestibility of the mopanie worm (*Imbrasia belina*). *Journal of Arid Environments* 68: 492-500.
- Makkar, H.P.S., Tran, G., Heuze, V. and Ankers, P., 2014: State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology* 197: 1-33.
- Maroni, G. and Watson, D., 1985. Uptake and binding of cadmium, copper and zinc by *Drosophila melanogaster* larvae. *Insect Biochemistry* 15: 55-63.
- Marono, S., Piccolo, G., Loponte, R., Di Meo, C., Attia, Y.A., Nizza, A. and Bovera, F., 2015. *In vitro* crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* insect meals and its correlation with chemical composition traits. *Italian Journal of Animal Sciences* 14: 338-343.
- Maryanski, M., Kramarz, P., Laskowski, R. and Niklinska, M., 2002. Decreased energetic reserves, morphological changes and accumulation of metals in carabid beetles (*Poecilus cupreus* L.) exposed to zinc-or cadmium-contaminated food. *Ecotoxicology* 11: 127-139.
- Massie, H.R., Williams, T.R. and DeWolfe, L.K., 1989. Changes in taurine in aging fruit flies and mice. *Experimental Gerontology* 24: 57-65.
- McComb, A., 2010. Evaluation of vitamin A supplementations for captive amphibian species. MSc thesis, North Carolina State University, Raleigh, North Carolina, 129 pp.
- Megido, R.C., Poelaert, C., Ernens, M., Liotta, M., Blecker, C., Danthine, S., Tyteca, E., Haubruge, E., Alabi, T., Bindelle, J. and Francis, F., 2018. Effect of household cooking techniques on the microbiological load and the nutritional quality of mealworms (*Tenebrio molitor* L. 1758). *Food Research International* 106: 503-508.
- Mlček, J., Adámková, A., Adámek, M., Borkovcová, M., Bednářová, M. and Knížková, I., 2019. Fat from Tenebrionidae bugs – sterols content, fatty acid profiles, and cardiovascular risk indexes. *Polish Journal of Food Nutrition Sciences* 69: 247-254
- Moussian, B., 2010. Recent advances in understanding mechanisms of insect cuticle differentiation. *Insect Biochemistry and Molecular Biology* 40: 363-375.
- Mwangi, M.N., Oonincx, D.G.A.B., Stouten, T., Veenbos, M., Melse-Boonstra, A., Dicke, M. and Van Loon, J.J., 2018. Insects as sources of iron and zinc in human nutrition. *Nutrition Research Reviews* 31: 248-255.
- Nakagaki, B.J., Sunde, M.L. and Defoliart, G.R., 1987. Protein quality of the house cricket, *Acheta domesticus*, when fed to broiler chicks. *Poultry Science* 66: 1367-1371.
- National Research Council (NRC), 1994. Nutrient requirements of poultry. Ninth Edition. National Academy Press, Washington, DC, USA, 157 pp.
- National Research Council (NRC), 2011. Nutrient requirements of fish and shrimp. National Academy Press, Washington, DC, USA, 376 pp.
- National Research Council (NRC), 2012. Nutrient requirements of Swine. Eleventh Edition. National Academy Press, Washington, DC, USA, 420 pp.
- Nestel, D., Papadopoulos, N.T., Liedo, P., Gonzales-Ceron, L. and Carey, J.R., 2005. Trends in lipid and protein contents during medfly aging: An harmonic path to death. *Archives of Insect Biochemistry and Physiology* 60: 130-139.
- Newbrey, J.L., Paszkowski, C.A. and Dumenko, E.D., 2013. A comparison of natural and restored wetlands as breeding bird habitat using a novel yolk carotenoid approach. *Wetlands* 33: 471-482.
- Nishimune, T., Watanabe, Y., Okazaki, H. and Akai, H., 2000. Thiamin is decomposed due to *Anopheles* spp. entomophagy in seasonal ataxia patients in Nigeria. *Journal of Nutrition* 130: 1625-1628.
- Noland, J.L. and Baumann, C.A. 1949. Requirement of the German cockroach for choline and related compounds. *Experimental Biology and Medicine* 70: 198-201.
- Nyangena, D.N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., Nakimbugwe, D. and Fiaboe, K.K., 2020. Effects of traditional processing techniques on the nutritional and microbiological quality of four edible insect species used for food and feed in East Africa. *Foods* 9: 574.
- Olson, J.A., 1989. Provitamin A function of carotenoids: the conversion of beta-carotene into vitamin A. *Journal of Nutrition* 119: 105-108.
- Omotoso, O.T. 2006. Nutritional quality, functional properties and anti-nutrient compositions of the larva of *Cirina forda* (Westwood) (Lepidoptera: Saturniidae). *Journal of Zhejiang University Science B* 7: 51-55.
- Onifade, A.A., Oduguwa, O.O., Fanimu, A.O., Abu, A.O., Olutunde, T.O., Arije, A. and Babatunde, G.M., 2001. Effects of supplemental methionine and lysine on the nutritional value of housefly larvae meal (*Musca domestica*) fed to rats. *Bioresource Technology* 78: 191-194.
- Oonincx, D.G.A.B. and Dierenfeld, E.S., 2012. An investigation into the chemical composition of alternative invertebrate prey. *Zoo Biology* 31: 40-54.
- Oonincx, D.G.A.B. and Van der Poel, A.F., 2011. Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). *Zoo Biology* 30: 9-16
- Oonincx, D., Bosch, G., Van Der Borght, M., Smets, R., Gasco, L., Fascetti, A., Yu, Z., Johnson, V., Tomberlin, J.K. and Finke, M., 2019b. A cross-laboratory study on analytical variability of amino acid content in three insect species. In: Book of Abstracts of the 70<sup>th</sup> Annual Meeting of the European Federation of Animal Science. Wageningen Academic Publishers, Wageningen, the Netherlands, p. 327.
- Oonincx, D.G.A.B., Laurent, S., Veenbos, M.E. and Van Loon, J.J.A., 2019a. Dietary enrichment of edible insects with omega 3 fatty acids. *Insect Science* 27: 500-509. <https://doi.org/10.1111/1744-7917.12669>
- Oonincx, D.G.A.B., Stevens, Y., Van den Borne, J.J.G.C., Van Leeuwen, J.P.T.M. and Hendriks, W.H., 2010. Effects of vitamin D3 supplementation and UVb exposure on the growth and plasma concentration of vitamin D3 metabolites in juvenile bearded dragons (*Pogona vitticeps*). *Comparative Biochemistry and Physiology B* 156: 122-128.

- Oonincx, D.G.A.B., Van Broekhoven, S., Van Huis, A. and Van Loon, J.J.A., 2015a. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS ONE* 10: e0144601.
- Oonincx, D.G.A.B., Van Keulen, P., Finke, M.D., Baines, F.M., Vermeulen, M. and Bosch, G., 2018. Evidence of vitamin D synthesis in insects exposed to UVb light. *Scientific Reports* 8 10807.
- Oonincx, D.G.A.B., Van Leeuwen, J.P., Hendriks, W.H. and Van der Poel, A.F.B., 2015b. The diet of free-roaming Australian central bearded dragons (*Pogona vitticeps*). *Zoo Biology* 34: 271-277.
- Ortiz, C., Weiss-Penzias, P.S., Fork, S. and Flegal, A.R., 2015. Total and monomethyl mercury in terrestrial arthropods from the central California coast. *Bulletin of environmental contamination and toxicology* 94: 425-430.
- Ottoboni, M., Spranghers, T., Pinotti, L., Baldi, A., De Jaeghere, W. and Eeckhout, M., 2018. Inclusion of *Hermetia illucens* larvae or prepupae in an experimental extruded feed: process optimisation and impact on *in vitro* digestibility. *Italian Journal of Animal Science* 17: 418-427.
- Oyarzun, S.E., Crawshaw, G.J. and Valdes, E.V., 1996. Nutrition of the tamandua: I. Nutrient composition of termites (*Nasutitermes spp.*) and stomach contents from wild tamanduas (*Tamandua tetradactyla*). *Zoo Biology* 15: 509-524.
- Ozimek, L., Sauer, W.C., Kozikowski, V., Ryan, J.K., Jorgensen, H. and Jelen, P., 1985. Nutritive value of protein extracted from honey bees. *Journal of Food Science* 50: 1327-1329.
- Paoletti, M.G., Norberto, L., Damini, R. and Musumeci, S., 2007. Human gastric juice contains chitinase that can degrade chitin. *Annals of Nutrition and Metabolism* 51: 244-251.
- Paul, A., Frederich, M., Megido, R.C., Alabi, T., Malik, P., Uyttenbroeck, R., Francis, F., Blecker, C., Haubruge, E., Lognay, G. and Danthine, S., 2017. Insect fatty acids: a comparison from three Orthopteran and *Tenebrio molitor* L. larvae. *Journal of Asia-Pacific Entomology* 20: 337-240.
- Payne, C.L.R., Scarborough, P., Rayner, M. and Nonaka, K., 2016. A systematic review of nutrient composition data available for twelve commercially available edible insects and comparison with reference values. *Trends in Food Science and Technology* 47: 69-77.
- Payne, C.L.R., Umemura, M., Dube, S., Azuma, A., Takenaka, C. and Nonaka, K., 2015. The mineral composition of five insects as sold for human consumption in Southern Africa. *African Journal of Biotechnology* 14: 2443-2448.
- Pearincott, J.V., 1960. Changes in lipid content during growth and metamorphosis of the house fly *Musca domestica* Linnaeus. *Journal of Cellular and Comparative Physiology* 55: 167-174.
- Pennino, M., Dierenfeld, E.S. and Behler, J.L., 1991. Retinol, alpha-tocopherol, and proximate nutrient composition of invertebrates used as feed. *International Zoo Yearbook* 30: 143-149.
- Pesti, G.M., Harper, A.E. and Sunde, M.L., 1979. Sulfur amino acid and methyl donor status of corn-soy diets fed to starting broiler chicks and turkey poults. *Poultry Science* 58: 1541-1547.
- Phelps, R.J., Struthers, J.K. and Moyo, S.J., 1975. Investigations into the nutritive value of *Macrotermes falciger* (Isoptera: Termitidae). *Zoologica Africana* 10: 123-132.
- Pieterse, E. and Pretorius, Q., 2014. Nutritional evaluation of dried larvae and pupae meal of the housefly (*Musca domestica*) using chemical and broiler-based biological assays. *Animal Production Science* 54: 347-355.
- Poelaert, C., Despret, X., Sindic, M., Beckers, Y., Francis, F., Portetelle, D., Soyeurt, H., Théwis, A. and Bindelle, J., 2017. Cooking has variable effects on the fermentability in the large intestine of the fraction of meats, grain legumes, and insects that is resistant to digestion in the small intestine in an *in vitro* model of the pig's gastrointestinal tract. *Journal of Agricultural and Food Chemistry* 65: 435-444.
- Poelaert, C., Francis, F., Alabi, T., Caparros Megido, R., Crahay, B., Bindelle, J. and Beckers, Y., 2018. Protein value of two insects, subjected to various heat treatments, using growing rats and the protein digestibility-corrected amino acid score. *Journal of Insects as Food and Feed* 4: 77-87. <https://doi.org/10.3920/JIFF2017.0003>
- Postma, J.F., VanNugteren, P. and De Jong, M.B.B., 1996. Increased cadmium excretion in metal-adapted populations of the midge *Chironomus riparius* (Diptera). *Environmental Toxicology and Chemistry: An International Journal* 15: 332-339.
- Proc, K., Bulak, P., Wiącek, D. and Bieganski, A., 2020. *Hermetia illucens* exhibits bioaccumulative potential for 15 different elements – implications for feed and food production. *Science of the Total Environment* 723: 138125.
- Punzo, F., 2003. Nutrient composition of some insects and arachnids. *Florida Scientist* 66: 84-98.
- Purschke, B., Scheibelberger, R., Axmann, S., Adler, A. and Jäger, H., 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain. *Food Additives and Contaminants: Part A* 34: 1410-1420.
- Ramos-Elorduy, J., Gonzalez, E.A., Hernandez, A.R. and Pino, J.M., 2002. Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. *Journal of Economic Entomology* 95: 214-220.
- Ramsay, S.L. and Houston, D.C., 2003. Amino acid composition of some woodland arthropods and its implications for breeding tits and other passerines. *Ibis* 145: 227-232.
- Rao, P.U., 1994. Chemical composition and nutritional evaluation of spent silk worm pupae. *Journal Agriculture and Food Chemistry* 42: 2201-2203.
- Raubenheimer, D. and Rothman, J.M. 2013. Nutritional ecology of entomophagy in humans and other primates. *Annual Review of Entomology* 58: 141-160.
- Reichle, D.E., Shanks, M.H. and Crossley, D.A., 1969. Calcium, potassium, and sodium content of forest floor arthropods. *Annals of the Entomological Society of America* 62: 57-62.
- Rho, M.S. and Lee, K.P., 2014. Geometric analysis of nutrient balancing in the mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *Journal of Insect Physiology* 71: 37-45.
- Riddick, E.W., 2014. Insect protein as a partial replacement for fishmeal in the diets of juvenile fish and crustaceans. In: Morales-Ramos, J.A., Rojas, M.G. and Shapiro, D.A. (eds) Mass production of beneficial organisms invertebrates and entomopathogens. Elsevier, Waltham, MA, USA, pp 565-582.

- Roe, R.M., Clifford, C.W. and Woodring, J.P., 1980. The effect of temperature on feeding, growth, and metabolism during the last larval stadium of the female house cricket, *Acheta domesticus*. *Journal of Insect Physiology* 26: 639-644.
- Roe, R.M., Clifford, C.W. and Woodring, J.P., 1985. The effect of temperature on energy distribution during the last-larval stadium of the female house cricket, *Acheta domesticus*. *Journal of Insect Physiology* 31: 371-378.
- Roseland, C.R., Grodowitz, M.J., Kramer, K.J., Hopkins, T.L. and Broce, A.B., 1985. Stabilization of mineralized and sclerotized puparial cuticle of muscid flies. *Insect Biochemistry* 15: 521-528.
- Rudel, L.L., Parks, J.S., Hedrick, L., Thomas, M. and Willifors, K. 1998. Lipoprotein and cholesterol metabolism in diet-induced coronary artery atherosclerosis in primates. Role of cholesterol and fatty acids. *Progress in Lipid Research* 37: 353-370.
- Rumpold, B.A. and Schluter, O.K., 2013a. Nutritional composition and safety aspects of edible insects. *Molecular Nutrition Food Research* 57: 802-823.
- Rumpold, B.A. and Schluter, O.K., 2013b. Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies* 17: 1-11.
- Sabolová, M., Adámková, A., Kouřimská, L., Chrpová, D. and Pánek, J., 2016. Minor lipophilic compounds in edible insects. *Potravinárstvo* 10: 400-406.
- Sanchez-Muros, M.J., Barroso, F.G. and De Haro, C., 2016. Brief summary of insect usage as an industrial animal feed/feed ingredient. In: Dorsey, A.T., Morales-Ramos, J.A. and Rojas, M.G. (ed) *Insects as sustainable food ingredients production, processing and food applications*. Elsevier, Waltham, MA, USA, pp 273-309.
- Sanchez-Muros, M.J., Barroso, F.G. and Manzano-Agugliaro, F., 2014. Insect meal as renewable source of food for animal feeding: a review. *Journal of Cleaner Production* 65: 16-27.
- Santos Oliveira, J.F., Passos De Carvalho, J., Bruno De Sousa, R.F.X. and Simao, M.M., 1976. The nutritional value of four species of insects consumed in Angola. *Ecology of Food and Nutrition* 5: 91-97.
- Schaefer, C.H., 1968. The relationship of the fatty acid composition of *Heliothis zea* larvae to that of its diet. *Journal of Insect Physiology* 14: 171-178.
- Schiavone, A., De Marco, M., Martínez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., Gai, F. and Gasco, L., 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology* 8: 51. <https://doi.org/10.1186/s40104-017-0181-5>
- Schmitt, E., Belghit, I., Johansen, J., Leushuis, R., Lock, E.J., Melsen, D., Shanmugam, R.K.R., Van Loon, J. and Paul, A., 2019. Growth and safety assessment of feed streams for black soldier fly larvae: a case study with aquaculture sludge. *Animals* 9: 189. <https://doi.org/10.3390/ani9040189>
- Seki, T., Isono, K., Ozaki, K., Tsukahara, Y., Shibata-Katsuta, Y., Ito, M., Irie, T. and Katagiri, M., 1998. The metabolic pathway of visual pigment chromophore formation in *Drosophila melanogaster* all-trans (3S)-3-hydroxyretinal is formed from all-trans retinal via (3R)-3-hydroxyretinal in the dark. *European Journal of Biochemistry* 257: 522-527.
- Shin, C.S., Kim, D.Y. and Shin, W.S., 2019. Characterization of chitosan extracted from mealworm beetle (*Tenebrio molitor*, *Zophobas morio*) and rhinoceros beetle (*Allomyrina dichotoma*) and their antibacterial activities. *International Journal of Biological Macromolecules* 125: 72-77.
- Sonmez, E. and Gulel, A., 2008. Effects of different temperatures on the total carbohydrate, lipid and protein amounts of the bean beetle, *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). *Pakistan Journal of Biological Sciences* 11: 1803-1808.
- Sprangers, T., Ottoboni, M., Klootwijk, C., Olyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. and De Smet, S., 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture* 97: 2594-2600.
- Ssepuyua, G., Mukisa, I.M. and Nakimbugwe, D., 2017. Nutritional composition, quality, and shelf stability of processed *Ruspolia nitidula* (edible grasshoppers). *Food Science & Nutrition* 5: 103-112.
- St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. and Irving, S., 2007. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *Journal of the World Aquaculture Society* 38: 309-313.
- Starčević, K., Gavrilović, A., Gottstein, Z. and Mašek, T., 2017. Influence of substitution of sunflower oil by different oils on the growth, survival rate and fatty acid composition of Jamaican field cricket (*Gryllus assimilis*). *Animal Feed Science and Technology* 228: 66-71.
- Strobel, S., Roswag, A., Becker, N.I., Trenczek, T.E. and Encarnação, J.A., 2013. Insectivorous bats digest chitin in the stomach using acidic mammalian chitinase. *PLoS ONE* 8: e72770.
- Studier, E.H. and Sevick, S.H., 1992. Live mass, water content, nitrogen and mineral levels in some insects from south-central lower Michigan. *Comparative Biochemistry and Physiology A* 103: 579-595.
- Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R. and Khanal, S.K. 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renewable Energy* 98: 197-202.
- Sushchik, N.N., Gladyshev, M.I., Moskvichova, A.V., Makhutova, O.N. and Kalachova, G.S. 2003. Comparison of fatty acid composition in major lipid classes of the dominant benthic invertebrates of the Yenisei river. *Comparative Biochemistry and Physiology B* 134: 111-122.
- Sushchik, N.N., Yurchenko, Y.A., Gladyshev, M.I., Belevich, O.E., Kalachova, G.S. and Kolmakova, A.A., 2013. Comparison of fatty acid contents and composition in major lipid classes of larvae and adults of mosquitoes (Diptera: Culicidae) from a steppe region. *Insect Science* 20: 585-600.



- Svoboda, J., Schiff, N. and Feldlaufer, M., 1995. Sterol composition of three species of sawflies (Hymenoptera: Symphyta) and their dietary plant material. *Experientia* 51: 150-152.
- Tabata, E., Kashimura, A., Kikuchi, A., Masuda, H., Miyahara, R., Hiruma, Y., Wakita, S., Ohno, M., Sakaguchi, M., Sugahara, Y., Matoska, V., Bauer, P.O. and Oyama, F., 2018. Chitin digestibility is dependent on feeding behaviors, which determine acidic chitinase mRNA levels in mammalian and poultry stomachs. *Scientific Reports* 8: 1461. <https://doi.org/10.1038/s41598-018-19940-8>
- Teffo, L.S., Toms, R.B. and Eloff, J.N., 2007. Preliminary data on the nutritional composition of the edible stink-bug, *Encosternum delegorguei* Spinola, consumed in Limpopo province, South Africa. *South African Journal of Science* 103: 434-436
- Teotia, J.S. and Miller, B.F., 1973. Fly pupae as a dietary ingredient for starting chicks. *Poultry Science* 52: 1830-1835.
- Teotia, J.S. and Miller, B.F., 1974. Nutritive content of house fly pupae and manure residue. *British Poultry Science* 15: 177-182.
- Thompson, S.N., 1973. Review and comparative characterization of fatty-acid compositions of seven insect orders. *Comparative Biochemistry and Physiology* 45: 467-482.
- Tietz, A. and Stern, N., 1969. Stearate desaturation by microsomes on the locust fat-body. *FEBS Letters* 2: 286-288.
- Timmermans, K.R. and Walker, P.A., 1989. The fate of trace metals during the metamorphosis of chironomids (Diptera, Chironomidae). *Environmental pollution* 62: 73-85.
- Tschirner, M. and Simon, A., 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed* 1: 249-259. <https://doi.org/10.3920/JIFF2014.0008>
- Ueckert, D.N., Yang, S.P. and Albin, R.C. 1972. Biological value of rangeland grasshoppers as a protein concentrate. *Journal of Economic Entomology* 65: 1286-1288.
- Van Broekhoven, S., Ooninx, D.G.A.B., Van Huis, A. and Van Loon, J.J.A., 2015. Growth performance and feed efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. *Journal of Insect Physiology* 73: 1-10.
- Van der Fels-Klerx, H.J., Camenzuli, L., Van der Lee, M.K. and Ooninx, D.G.A.B., 2016. Uptake of cadmium, lead and arsenic by *Tenebrio molitor* and *Hermetia illucens* from contaminated substrates. *PLoS ONE* 11: e0166186.
- Vijver, M., Jager, T., Posthuma, L. and Peijnenburg, W., 2003. Metal uptake from soils and soil-sediment mixtures by larvae of *Tenebrio molitor* (L.)(Coleoptera). *Ecotoxicology and Environmental Safety* 54: 277-289.
- Vogt, K. and Kirschfeld, K., 1984. Chemical identity of the chromophore of fly visual pigment. *Naturwissenschaften* 71: 211-213.
- Von Lintig, J., 2012. Metabolism of carotenoids and retinoids related to vision. *Journal of Biological Chemistry* 287: 1627-1634.
- Wang, D., Zhai, S.W., Zhang, C.X., Bai, Y.Y., An, S.H. and Xu, Y.N., 2005. Evaluation on nutritional value of field crickets as a poultry feedstuff. *Asian-Australasian Journal of Animal Sciences* 18: 667-670.
- Wang, D., Zhai, S.W., Zhang, C.X., Zhang, Q. and Chen, H., 2007. Nutrition value of the Chinese grasshopper *Acrida cinerea* (Thunberg) for broilers. *Animal Feed Science and Technology* 135: 66-74.
- Wang, S. Y., Wu, L., Li, B. and Zhang, D., 2020. Reproductive potential and nutritional composition of *Hermetia illucens* (Diptera: Stratiomyidae) prepupae reared on different organic wastes. *Journal of Economic Entomology* 113: 527-537.
- Whitaker, Jr., J.O., Dannelly, H.K. and Prentice, D.A., 2004. Chitinase in insectivorous bats. *Journal of Mammalogy* 85: 15-18.
- Whitton, P.S., Strang, R.H.C. and Nicholson, R.A., 1987. The distribution of taurine in the tissues of some species of insects. *Insect Biochemistry* 17: 573-577.
- WHO/FAO/UNU Expert Consultation, 2007. Protein and amino acid requirements in human nutrition. WHO Technical Reports Series 935. World Health Organization, Geneva, Switzerland.
- Woods, M.J., Goosen, N.J., Hoffman, L.C. and Pieterse, E., 2020. A simple and rapid protocol for measuring the chitin content of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae. *Journal of Insects as Food and Feed* 6: 285-290. <https://doi.org/10.3920/JIFF2019.0030>
- Wu, G.X., Ye, G.E., Hu, C. and Cheng, J.A., 2006. Accumulation of cadmium and its effects on growth, development and haemolymph biochemical compositions in *Boettcherisca peregrina* larvae (Diptera: Sarcophagidae). *Insect Science* 13: 31-39.
- Wu, N., Wang, X., Xu, X., Cai, R. and Xie, S., 2020. Effects of heavy metals on the bioaccumulation, excretion and gut microbiome of black soldier fly larvae (*Hermetia illucens*). *Ecotoxicology and Environmental Safety* 192: 110323.
- Xia, Z., Wu, S., Pan, S. and Kim, J.M., 2012. Nutritional evaluation of protein from *Clanis bilineata* (Lepidoptera), an edible insect. *Journal of the Science of Food and Agriculture* 92: 1479-1482.
- Yang, L.F., Siriamornpun, S. and Li, D., 2006. Polyunsaturated fatty acid content of edible insects in Thailand. *Journal of Food Lipids* 13: 277-285.
- Yi, L., Lakemond, C.M.M., Sagis, L.M.C., Eisner-Schadler, V., Van Huis, A. and Van Boekel, M.A.J.S., 2013. Extraction and characterisation of protein fractions from five insect species. *Food Chemistry* 141: 3341-3348.
- Zhang, Z., Song, X., Wang, Q. and Lu, X., 2012. Cd and Pb contents in soil, plants, and grasshoppers along a pollution gradient in Huludao City, Northeast China. *Biological Trace Element Research* 145: 403-410.
- Zhang, Z.-S., Lu, X.-G., Wang, Q.-C. and Zheng, D.-M., 2009. Mercury, cadmium and lead biogeochemistry in the soil-plant-insect system in Huludao City. *Bulletin of environmental contamination and toxicology* 83: 255.
- Zhou, J.R. and Erdman, J.W., 1995. Phytic acid in health and disease. *Critical Reviews in Food Science and Nutrition* 35: 495-508.
- Zhou, X., Honek, A., Powell, W. and Carter, N., 1995. Variations in body length, weight, fat content and survival in *Coccinella septempunctata* at different hibernation sites. *Entomologia Experimentalis et Applicata* 75: 99-107.
- Zhuang, P., Huiling, Z. and Wensheng, S., 2009. Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: field study. *Journal of Environmental Sciences* 21: 849-853.

