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 β -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*).¹

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

¹ 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 subhyalinus*) 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

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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.¹

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
¹ The amino acid data shown for insects are an average of data from published sources (Finke, 2002, 2007, 2015b; Spranghers <i>et al.</i> , 2017; Yi <i>et al.</i> , 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.¹

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)
	<i>O. rhinoceros</i> larvae	90.1	boiled	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)
	<i>I. belina</i> larvae meal	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)
Orthoptera	<i>Clanis bilineata</i> larvae meal	95.8	heads removed and dried at 60 °C	96.1	Xia <i>et al.</i> (2012)
	<i>Acheta domesticus</i> meal	83.9	freeze dried	98.8	Poelaert <i>et al.</i> (2018)

¹ * = 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- β -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; Sprangers *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; Sprangers *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 (Cerdeña *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 β -carotene to species that use retinal as their chromophore such as house crickets, yellow mealworm larvae, and superworm larvae increases β -carotene concentration in their tissues (Finke, 2015a). Providing a diet containing β -carotene to species that use 3-hydroxyretinal as their chromophore such as Diptera and Lepidoptera typically does not increase β -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 β -carotene when the larvae were fed diets containing β -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 (Ssepuuya *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, β -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.

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