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Mass production of beneficial organisms

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Chapter 18

Insects as food for insectivores

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18.1 Introduction

Insects are generally considered a good source of most nutrients (DeFoliart, 1992) and many species have been analyzed for their nutrient composition. These analyses include insects taken from the field (Bukkens, 1997; Finke, 2015b; Gullan and Cranston, 2005; Punzo, 2003) and those commercially reared (Dierenfeld and King, 2008; Finke, 2002, 2013, 2015a; Oonincx et al., 2010; Oonincx and Dierenfeld, 2012; Ramos-Elorduy et al., 2002; Simpson and Raubenheimer, 2001). In nature, most insectivores consume insects and a variety of other arthropods including arachnids (scorpions, whipscorpion, ticks, and spiders), isopods, millipedes and centipedes. As a general rule, it is better for insectivores to be offered a varied diet rather than a single insect species as a mixed diet is less likely to result in nutrient deficiencies.

A number of variables can influence the chemical composition of insects, such as gender (Ali and Ewiess, 1977; Hoffmann, 1973; Sonmez and Gulel, 2008), stage of development (McClements et al., 2003), diet (Calvez, 1975; Oonincx and van der Poel, 2011; Ramos-Elorduy et al., 2002; Simpson and Raubenheimer, 2001) and environmental factors such as temperature (Hoffmann, 1973; Sonmez and Gulel, 2008), day length (Ali and Ewiess, 1977; Koc and Gulel, 2008; Shearer and Jones, 1996), light intensity and spectral composition (Oonincx et al., 2018) and humidity (Ali et al., 2011; Han et al., 2008; Nedvěd and Kalushkov, 2012).

Nutrient densities can be expressed based on the fresh or dry weight. As insectivores consume live prey, fresh weight is sometimes preferred. While water is a critical nutrient, in most cases providing it is not the primary function of offering insects as food. Furthermore, variation in moisture content strongly influences the nutrient density when expressed as fresh weight. This hampers a thorough nutritional comparison. Therefore, in this chapter nutrient densities are expressed on a dry matter basis. As indicated, large variations in dry matter content exist between species and developmental stages. As a rule of thumb, the dry matter content typically ranges from 13% to 42% of the fresh weight of a live insect (Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002, 2013; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Punzo, 2003).

18.2 Nutrient content of insects

18.2.1 Protein and amino acids

The protein content of insects is highly variable and ranges between 7.5% and 91% with many species containing around 60% protein on a dry matter basis (Barker et al., 1998; Bernard and Allen, 1997; Bukkens, 1997; Finke, 2002, 2013; Oonincx et al., 2010; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Oonincx et al., 2015; Punzo, 2003; Ramos-Elorduy et al., 2002). The protein content is commonly determined by multiplying the amount of nitrogen times 6.25, known as the crude protein content. This factor is underestimated if not all amino acids are properly quantified (Oonincx et al., 2019). Conversely, the presence of nonprotein nitrogen from compounds, such as chitin, uric acid, melanin and β-alanine, leads to overestimations of true protein content when using this factor. An alternative Kp of 4.76 for insects has been suggested based on amino acid data from only three species of insect larva analyzed by a single laboratory (Janssen et al., 2017). Other authors suggest Kp ranges of 4.21−5.05 and 5.25−5.33 for five insect species while reporting varying values for the same species and life stage (Belghit et al., 2019; Boulos et al., 2020). Recalculating the data from an even wider set of 20 insect samples, including 13 species and different developmental stages results in an average Kp of 5.81; ranging from 4.56 to 6.45 (Finke, 2002, 2007, 2013, 2015a, 2015b). 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 is recommended to facilitate comparisons between studies.

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Amino acids are the building blocks of proteins. Certain amino acids are known as essential amino acids because they cannot be synthesized from simpler molecules by most animal species (Bender, 2002). It seems likely that the amino acid composition of insects (expressed as mg/g protein) is fairly constant within a given species and life stage. Wings, legs, mandibles, and other body parts have specific physical requirements in order to function properly; therefore, differences in amino acid composition between insects fed different diets are unlikely. Prepupae from black soldier fly larvae (*Hermetia illucens* L.) fed different diets had similar amino acid patterns (Lalander et al., 2019; Spranghers et al., 2017; Wang et al., 2020), as did tobacco hornworm larvae (*Manduca sexta* (L.)) fed two different diets (Landry et al., 1986). Additionally, a review of house cricket (*Acheta domesticus* L.) amino acid composition (expressed as mg of amino acids/g protein) from six different papers showed very similar amino acid patterns (Oonincx and Finke, 2021).

Prediction of an insect's amino acid profile based on species, age, and life stage is difficult at best. Table 18.1 shows the amino acid pattern of six commonly raised insect species and compares them to the amino acid requirements of rats, growing broiler chicks (poultry) and trout (NRC, 1994, 1995, 2011).

TABLE 18.1 Typical amino acids patterns (mg/g crude protein) of some common feeder insects and amino acid scores and first limiting amino acid for various species.

	Acheta domesticus	*Tenebrio molitor* larvae	*Zophobas mori* larvae	*Hermetia illucens* larvae/prepuape	*Blatta lateralis* nymphs	*Bombyx mori* larvae
Alanine	87.9	80.2	72.7	62.7	87.9	44.6
Arginine	65.7	60.0	57.4	52.8	79.3	65.6
Aspartic acid	79.1	81.0	83.1	88.3	73.9	41.4
Glutamic acid	109.2	112.1	127.0	103.7	118.8	99.5
Glycine	52.3	53.1	48.7	55.0	65.2	59.7
Histidine	22.8	30.2	31.1	32.4	28.9	25.8
Isoleucine	40.3	46.1	46.9	43.3	40.7	32.3
Leucine	78.6	84.9	80.4	69.9	63.0	52.7
Lysine	55.3	55.4	54.6	59.1	67.2	46.8
Methionine	15.8	13.3	12.2	18.8	17.6	13.4
Methionine + cystine	24.9	23.3	21.8	24.4	25.2	22.0
Phenylalanine	31.5	35.2	37.2	41.5	40.4	28.5
Phenylalanine + tyrosine	87.5	102.6	108.4	112.3	115.6	59.7
Proline	56.7	68.1	55.9	55.4	55.9	33.9
Serine	44.1	47.2	44.1	38.3	44.1	36.0
Threonine	35.7	40.3	39.9	39.0	41.5	30.6
Tryptophan	7.2	10.5	11.4	15.1	8.7	7.0
Valine	54.1	62.9	60.5	63.8	64.7	40.3
Taurine	6.1	0.4	ND	ND	ND	ND
Amino acids score/first limiting amino acid for						
Rats	38/ met + cys	36/met + cys	33/met + cys	37/met + cys	39/met + cys	34/met + cys
Poultry	64/ met + cys	60 met + cys	56/met + cys	62/met + cys	64/met + cys	56/met + cys
Trout	52/ met + cys	49/met + cys	46/met + cys	51/met + cys	53/met + cys	45/lys

The nutritional quality of insect protein has generally been described as good, but the quality depends on the digestibility of the amino acids and the match of the amino acid profile to the requirements of the insectivore in question (Finke et al., 1987, 1989; Ramos-Elorduy, 1997). Unfortunately, the amino acid requirements of most insectivores are unknown, so most comparisons have been made using more common laboratory animals, such as rats or chickens (Finke, 2002, 2013).

The first limiting amino acid depends on both the insect species being consumed and the species consuming the prey. Since different insectivore species likely require different proportions of amino acids, the first limiting amino acids could differ between species. In chickens fed purified diets where Mormon cricket (*Anabrus simplex* Haldeman) or house cricket meal was the sole source of protein, the first limiting amino acids were methionine and arginine (Finke et al., 1985; Nakagaki et al., 1987). When fed to growing rats, the first limiting amino acids in insect protein from yellow mealworm larvae (*Tenebrio molitor* L.), common housefly (*Musca domestica* L) larvae and adult Mormon crickets were methionine (Finke et al., 1987; Goulet et al., 1978; Onifade et al., 2001). Similarly, methionine and cystine were calculated to be the first limiting amino acids for rats in house crickets, yellow mealworm larvae, lessor mealworms (*Alphitobius diaperinus* (Panzer)), superworm larvae (*Zophobas morio* F.), larvae of the greater wax moth (*Galleria mellonella* L.), domesticated silkworm larvae (*Bombyx mori* L.), honey bee larvae and pupae (*Apis mellifera* L.), adult common houseflies, black soldier fly larvae, Turkestan roaches (*Blatta lateralis* Walker) and butter worm larvae (*Chilecomadia moorei* Silva) (Finke, 2002, 2013; Jensen et al., 2019; Poelaert et al., 2018). As such it seems likely that for most mammalian insectivores the sulfur amino acids are the first-limiting. For fish, birds and other species, especially those without a functioning urea cycle, other amino acids such as arginine, may be important.

18.2.2 Fats and fatty acids

The most common way to estimate insect fat content is by determining the total weight of all fat-soluble molecules (mostly lipids but also waxes and some other compounds). Fat tissue is used for energy storage in the body and is either obtained directly from the diet or synthesized from carbohydrates (Bender, 2002; Fast, 1970; Hanson et al., 1983). The main storage site for insect lipids is the fat body (Beenakkers et al., 1985). As a dietary component, fat is not only an important energy source but may also play a role in the palatability of the insect when fed to insectivores. Large variations in the lipid content (4.6%−64% dry matter) of insects have been reported (Barker et al., 1998; Bukkens, 1997; Finke, 2002, 2013; Punzo, 2003; Yang et al., 2006).

The behavioral ecology of a species likely influences the amount of fat stored (Thompson, 1973). In some species, males have a higher fat content than females (Fast, 1970; Nakasone and Ito, 1967). For species where male combat is customary (for instance Odonata), this can be explained by a need for greater energy reserves. In silkworms and certain Saturnids (Lepidoptera), males also have greater fat reserves than females (Beenakkers et al., 1985). Generally, however, females have greater fat reserves than males (Kulma et al., 2019; Lease and Wolf, 2011; Nestel et al., 2005; Zhou et al., 1995). Species that accumulate energy reserves for reproduction during their larval stages would have an increased fecundity potential, as eggs have a high lipid content (Beenakkers et al., 1985; Downer and Matthews, 1976; Lease and Wolf, 2011). Before oviposition, these females would have a higher fat content than after oviposition (Lipsitz and McFarlane, 1971; Nestel et al., 2005). Insects collected from the wild seem to have a lower fat content than insects which are commercially produced (Finke, 2002, 2013; Oonincx and Dierenfeld, 2012; Yang et al., 2006). This might be a result of decreased movement in captivity, easy access to high energy diets or a combination of the two.

Fatty acids are the building blocks of fat. Two or three fatty acids are coupled to glycerol to form diglycerides and triglycerides respectively. These fatty acids are stored in the insect fat body, making up over 90% of the total fat body lipid (Beenakkers et al., 1985; Bender, 2002; Downer and Matthews, 1976). Based on the degree of saturation, fatty acids can be subdivided into saturated fatty acids (those with no double bonds) and unsaturated fatty acids (those with one (mono-unsaturated fatty acids) or more (poly-unsaturated fatty acids) double bonds). Poly-unsaturated fatty acids can be further subdivided into omega 3, 6, or 9 unsaturated fatty acids based on the relative position of the first double bond. Both the absolute occurrence of unsaturated fatty acids (Haglund et al., 1998) and the relative occurrence of specific unsaturated fatty acids (Schmitz and Ecker, 2008) are associated with health in humans, and these proportions might also play a role in the health of some species of insectivores.

For most insect species, more than half of their fatty acids are unsaturated, a notable exception being the Hemiptera (Thompson, 1973). The main saturated fatty acids found in insects are C16:0 (palmitic acid) and C 18:0 (stearic acid). As is observed with most other land animals, C16:0 is normally present in larger quantities than C18:0 (Fast, 1970; Majumder and Sengupta, 1979; Thompson, 1973; Yang et al., 2006). The most prevalent unsaturated fatty acids found in insects are C16:1 (palmitoleic acid), C18:1 (oleic acid), C18:2 (linoleic acid), and C18:3 (linolenic acid) (Beenakkers et al., 1985; Bukkens, 1997; Cookman et al., 1984; Ekpo et al., 2009; Fast, 1970; Majumder and Sengupta, 1979; Yang et al., 2006).

The fatty acid composition of insects is affected by four main variables: (1) species, (2) developmental phase, (3) diet, and (4) environmental factors such as temperature, light, and humidity.

Phylogeny is not the main determining factor for fatty acid composition, although some general distinctions can be made (Fast, 1970; Fontaneto et al., 2011). For instance, aphids and other Hemiptera tend to contain large amounts of the short-chained fatty acids C12:0 (lauric acid), and C14:0 (myristic acid) (Fast, 1970; Thompson, 1973). Lepidopterans tend to have a higher C18:3 content than other insect species (Fast, 1970; Fontaneto et al., 2011; Majumder and Sengupta, 1979), while Dictyoptera, such as cockroaches, contain little or no C18:3 (Finke, 2013; Thompson, 1973). In most species of Diptera C16:0, C16:1, and 18:1 predominate although C18:2 is also present in high levels in gall midges (Cecidomyiidae) (Fast, 1970; Thompson, 1973). Black soldier fly larvae contain high levels (up to 79% of the total fatty acids) of C12:0 although the exact amounts are determined by the insect's diet and life stage (Danieli et al., 2019; Ewald et al., 2020; Giannetto et al., 2020a; Hoc et al., 2020; Liu et al., 2017; Meneguz et al., 2018; Oonincx et al., 2019; Spranghers et al., 2017; St-Hilaire et al., 2007). Of note is that lauric acid has antimicrobial properties and the inclusion of black soldier fly fat in place of soybean oil into diets for young turkeys reduces the proliferation of potentially pathogenic bacteria (Sypniewski et al., 2020).

The fatty acid patterns of aquatic and terrestrial insects differ presumably because of their diet. Aquatic insects have relatively high levels of long-chain omega-3 fatty acids, in particular, 20:5 (eicosapentaenoic acid) and 22:6 (docosahexaenoic acid), which are rarely found in terrestrial insects (Fontaneto et al., 2011; Ghioni et al., 1996; Sushchik et al., 2003, 2013).

Insects contain higher amounts of C18:3 than most mammals. Like vertebrates, de novo synthesis of saturated fatty acids and poly-unsaturated fatty acids by elongation and desaturation occurs in insects (Beenakkers et al., 1985, Tietz and Stern, 1969). Certain poly-unsaturated fatty acids such as C18:2 and C18:3 are required in the diet because most animal species, including most insects, are unable to synthesize them (Bender, 2002; Cookman et al., 1984; Fast, 1970; Thompson, 1973). A few noticeable exceptions have been identified, for example, the American cockroach (*Periplaneta americana* L.) and the house cricket (Beenakkers et al., 1985; Blomquist et al., 1991). Several studies have shown that C18:2 can be synthesized de novo in these and other insect species, distributed over four different orders (Blomquist et al., 1991; Borgeson et al., 1991; Borgeson and Blomquist, 1993; de Renobales et al., 1987).

18.2.3 Carbohydrates

Few publications have focused on the carbohydrate content of insects. In general, carbohydrates are present in relatively small amounts in insects (Finke, 2002, 2013). In the two-spotted field cricket (*Gryllus bimaculatus* De Geer), polysaccharide and carbohydrate content are approximately 0.3% of the fresh weight (Hoffmann, 1973) and free carbohydrate content of the fat body in females of this species is less than 0.5% dry matter (Lorenz and Anand, 2004). In yellow mealworm larvae, the carbohydrate content can vary between 1% and 7% dry matter, depending on the diet provided (Ramos-Elorduy et al., 2002) although most of these differences are likely the result of food remaining in the gastrointestinal tract.

18.2.4 Fiber and chitin

Insects contain significant amounts of fiber as measured by crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Barker et al., 1998; Finke, 1984, 2002, 2007, 2013; Lease and Wolf, 2010; Oonincx and Dierenfeld, 2012; Pennino et al., 1991; Punzo, 2003). While insects contain significant amounts, the components that make up these fibers are unknown. It has been suggested that the fiber in insects represents chitin, since chitin (linear polymer of β-(1→4) N-acetyl-D-glucosamine units) is structurally similar to cellulose (linear polymer of β-(1→4)-D-glucopyranose units) found in plant material. In reality the fiber in whole insects likely represents a variety of different compounds including chitin, sclerotized proteins and other substances that are bound to chitin (Finke, 2007). Chitinase activity has been reported in certain species of frogs, lizards, tortoises, fish, birds and mammals suggesting that certain insectivores might be able to digest chitin (Donoghue, 2006; Fujimoto et al., 2002; German et al., 2010; Jackson et al., 1992; Jeuniaux and Cornelius, 1978; Lindsay, 1984; Tabata et al., 2018; Whitaker et al., 2004).

In insects, chitin exists in a matrix with proteins, lipids, and other compounds which together comprise the cuticle (Kramer et al., 1995). This matrix makes it difficult to analyze pure chitin in insects and to provide good quantitative estimates. However, since chitin is present only in the insect's exocuticle its' concentration is likely low. Methods used to estimate insect chitin include a modified CF analysis (Liu et al., 2012; Woods et al., 2020), ADF (Barker et al., 1998), ADF corrected for residual amino acids (Finke, 2007, 2013; Giannetto et al., 2020a), digestion by sodium hydroxide (Lease and Wolf, 2010), glucosamine determination after the breakdown of chitin (Cauchie, 2002; Henriques et al., 2020) and calcofluor staining (Henriques et al., 2020). In most studied insects, protein, not chitin is the predominant compound in the cuticle (Kramer et al., 1995). The amino acid patterns from whole insects differ from their ADF fractions and these patterns in ADF fractions also differ between species (Finke, 2007). These differences likely reflect specificity in insect cuticular proteins, which contribute to their unique properties.

While "harder bodied" insects like adult beetles contain higher levels of ADF than softer bodied insects like yellow mealworm larvae, silkworm larvae or cricket nymphs, those were due to higher levels of amino acids in the ADF fraction (Finke, 2007). This suggests that insects with "harder" cuticles do not necessarily contain more chitin than softer bodied insects, but rather contain higher levels of cross-linking proteins that are essential for sclerotization.

Some plant-based fibers can function as prebiotics inhibiting the growth of pathogenic bacteria and promoting the growth of beneficial ones. Little is known regarding the effects of fiber from insects on insectivores, although some research suggests pigs, poultry and fish fed diets containing dried insect meals have improved gut microbiota or immune function (Bruni et al., 2018; Ringø et al., 2012; Schiavone et al., 2017; Spranghers, et al., 2018). The exact components in these meals responsible for these effects are however unknown.

18.2.5 Minerals

Minerals can be broadly classified as macro-minerals and micro- or trace minerals based on the amounts needed to meet requirements. The essential macrominerals include calcium, phosphorus, magnesium, sodium, potassium and chloride. The essential microminerals include iron, zinc, copper, manganese, iodine, and selenium. The macrominerals calcium, phosphorus and magnesium play a primary role in helping maintain the skeletal structure in vertebrates while sodium, potassium and chloride function as electrolytes and help maintain acid−base balance. The trace minerals play wide-ranging roles ranging from oxygen transport to functioning as cofactors in a variety of enzyme systems.

Most species of insects contain little calcium because insects, as invertebrates, do not have a mineralized skeleton. Calcium levels are typically less than 0.3% dry matter (Barker et al., 1998; Finke, 2002, 2013; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Punzo, 2003; Reichle et al., 1969; Studier and Sevick, 1992). The higher levels of calcium occasionally reported for feeder crickets likely reflect calcium in the gut contents (Barker et al., 1998; Finke, 2003; Hatt et al., 2003; Punzo, 2003). The exoskeleton of most insects is primarily composed of protein and chitin, although some insects have a mineralized exoskeleton in which calcium and other minerals are incorporated into the cuticle (Dashefsky et al., 1976). Examples include larvae of the face fly, *Musca autumnalis* De Geer, and the black soldier fly (Boykin et al., 2020; Dierenfeld and King, 2008; Finke, 2013). Calcium levels reported for commercially available black soldier fly larvae ranged from 2.14% to 3.14% with calcium:phosphorus ratios of 1.85 to 2.6:1 (Boykin et al., 2020; Dierenfeld and King, 2008; Finke, 2013). However, black soldier fly larvae fed various waste products were even more variable with calcium levels ranging from 0.84% to 8.29% dry matter and with calcium:phosphorus ratios of 1.1:1 to 4.7:1 (Liland et al., 2017; Tschirner and Simon, 2015). Therefore, reliable data on the calcium content of specific sources and batches is needed before black soldier fly larvae can be considered a reliable source of calcium for insectivores. Moreover, besides calcium content, its' ratio with phosphorus is of relevance to facilitate adequate bone mineralization in insectivores. Other invertebrates such as millipedes and isopods also have a mineralized exoskeleton and likely serve as a source of calcium for wild insectivores (Gist and Crossley, 1975; Graveland and van Gijzen, 1994; Oonincx and Dierenfeld, 2012; Reichle et al., 1969). Another way of supplying calcium might be offering calcium grit, powder, or other calcium rich materials separately to insectivores to allow self-selection (Classen and Scott. 1982; Rich and Talent, 2008; Tordoff, 2001). Wild birds seek out calcium-rich invertebrates when calcium requirements are high, such as during egg-laying and nestling growth (Graveland and van Gijzen, 1994; Bureš and Weidinger, 2003).

The phosphorus content of feeder insects is much higher than calcium levels in most insect species, except for black soldier fly larvae (Barker et al., 1998; Finke, 2002, 2013; Hatt et al., 2003; Jones et al., 1972; Martin et al., 1976; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Punzo, 2003). Most insects likely contain adequate levels of phosphorus to meet the requirements of insectivores. To what extent this phosphorus is available is unclear. Using tibia ash as the criteria for phosphorus availability relative to dicalcium phosphate, 92% of the phosphorus in dried face fly pupa (*M. autumnalis* DeGeer) was available when fed to poultry (Dashefsky et al., 1976). In contrast, only 24% of the phosphorus in a diet containing dried black soldier fly larva was absorbed when fed to newly weaned five-week-old pigs compared to 51% for a corn-soy diet (Newton et al., 1977). Of note is that the diet containing dried back soldier fly larvae also contained other ingredients (corn and dicalcium phosphate) which provided significant amounts of phosphorus making it impossible to definitively determine the phosphorus availability from the dried back soldier fly larvae.

Most species of feeder insects contain levels of magnesium ranging from 0.08% to 0.40% dry matter (Barker et al., 1998; Finke, 2002, 2013; Jones et al., 1972; Martin et al., 1976; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Punzo, 2003). These levels would likely meet the dietary requirements of most insectivore species. Like calcium, the magnesium content of black soldier fly larvae was 3−10 times higher than that of other feeder insects (Boykin et al., 2020; Dierenfeld and King, 2008; Finke, 2013). It seems likely that both calcium and magnesium form a complex with chitin in the larval cuticle of this species.

There are few reports on the sodium and potassium content of captive-bred insects (Finke, 2002, 2013, 2015a; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012), but these data are comparable to the values obtained for wild-caught insects (Finke, 2015b; Gist and Crossley, 1975; Levy and Cromroy, 1973; Oyarzun et al., 1996; Reichle et al., 1969; Studier et al., 1991; Studier and Sevick, 1992). Levels of potassium generally range from 0.6%−2.0% dry matter, while sodium levels are lower ranging from 0.1% to 0.6% dry matter. There are limited data concerning the chloride content of feeder insects with values ranging from 0.16% to 0.97% dry matter (Finke, 2002, 2013, 2015a, 2015b). These data suggest that most insects likely contain adequate amounts of these three minerals to meet the needs of most insectivore species (Finke, 2002, 2013; Oonincx and Dierenfeld, 2012).

Most insects appear to contain relatively high levels of the trace minerals iron, zinc, copper and manganese. Additionally, iron, copper, zinc, and manganese availability from grasshoppers (*Sphenarium purpurascens* Charpentier), two-spotted field crickets, yellow mealworm larvae, and lesser mealworm larvae as measured by an in vitro assay was higher than that observed for beef suggesting these minerals are likely readily available to insectivores (Latunde-Dada et al., 2016).

While the high-fat larval stage of some species of feeder insects like greater wax moth larvae, yellow mealworm larvae and butterworm larvae might be marginally low in iron, relative to energy content, most insects likely supply adequate amounts for the typical insectivore (Barker et al., 1998; Finke, 2002, 2013, 2015a; Hatt et al., 2003; Mwangi et al., 2018; Montowska et al., 2020; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Punzo, 2003). Both adult houseflies and fruit flies (*Drosophila melanogaster* Meigen) contain relatively high levels of iron (125−454 mg iron/kg dry matter) (Barker et al., 1998; Finke, 2013; Oonincx and Dierenfeld, 2012). House crickets can contain up to 200 mg iron/kg dry matter although other reports documented iron content to be between 60 and 80 mg/kg dry matter (Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002, 2015a). These variations might be due to food remaining in the gastro-intestinal tract when the insects were analyzed, although potential contamination during processing should also be considered. Wild-caught insects also appear to contain significant amounts of iron (Finke, 2015b; Levy and Cromroy, 1973; Punzo, 2003; Studier et al., 1991; Studier and Sevick, 1992).

Insects are generally a good source of zinc with values for commercially raised feeder insects ranging from 61.6 to 340.5 mg/kg dry matter (Barker et al., 1998; Finke, 2002, 2013, 2015a; Latunde-Dada et al., 2016; Montowska et al., 2020; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Punzo, 2003). These values are similar to those obtained for wild-caught species (Finke, 2015b; Punzo, 2003).

Copper in commercially raised feeder insects ranged from 3.1 to 51.2 mg/kg dry matter (Barker et al., 1998; Finke, 2002, 2013, 2015a; Latunde-Dada et al., 2016; Montowska et al., 2020; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Punzo, 2003). The lowest value seen in captive-raised insects was 3.1 mg copper/kg dry matter for greater wax moth larvae reported by Barker (Barker et al., 1998) while Finke (2002) found a much higher level for this species (9.2 mg copper/kg dry matter). All other species analyzed had values greater than 7 mg copper/kg dry matter suggesting insects are typically good sources of copper. Wild-caught insects also appear to contain significant amounts of copper (Finke, 2015b; Levy and Cromroy, 1973; Punzo, 2003).

Levels of manganese in commercially raised feeder insects range from 1.5 to 364 mg/kg dry matter (Barker et al., 1998; Bernard and Allen, 1997; Dierenfeld and King, 2008; Finke, 2002, 2013, 2015a; Latunde-Dada et al., 2016; Montowska et al., 2020; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012; Punzo, 2003). Like calcium and magnesium, the highest levels of manganese observed were seen in black soldier fly larvae (Boykin et al., 2020; Dierenfeld and King, 2008; Finke, 2013) although the reasons for elevated levels of manganese in this species are unclear. Several species of stored product insects contain elevated levels of zinc and manganese in their mandibles, presumably to enable them to penetrate whole seeds (Morgan et al., 2003). Wild-caught insects also appear to contain significant amounts of manganese (Finke, 2015b; Punzo, 2003).

There are very little data regarding the iodine content of insects. Of the twelve species of commercially raised feeder insects analyzed only six had detectable iodine with levels ranging from 0.45 to 1.22 mg/kg dry matter (Finke, 2002, 2013, 2015a). Black soldier fly larvae fed graded levels of iodine-rich (700 mg/kg dry matter) seaweed contained increasing levels of iodine (0−260 mg /kg dry matter) (Liland et al., 2017). Since these authors harvested fully fed larvae at least some of these increases are likely a result of the food remaining in the gastrointestinal tract. Bee brood (pupae and larvae) and wild-caught white-lined sphinx moths (*Hyles lineata* Fabricius) did not contain detectable levels of iodine while wild-caught pallid winged grasshoppers (*Trimerotropis pallidipennis* Burmeister) and rhinoceros beetles (*Oxygrylius ruginasus* LeConte) contained 0.89 and 0.46 mg I/kg dry matter respectively (Finke, 2005, 2015b).

As is the case for iodine, only limited data on the selenium content of commercially raised feeder insects are available. While butterworm larvae did not contain any selenium the other eleven species of commercially raised feeder insects contained selenium at levels ranging from 0.27 to 0.97 mg/kg dry matter (Finke, 2002, 2013, 2015a). Three species of wild caught insects contained similar levels (0.55−0.75 mg Se/kg dry matter) as those reported for commercially raised feeder insects (Finke, 2015b). In contrast, Indonesian sago grub (larvae of the beetle *Rhynchophorus bilineatus* Montrouzier) contained no detectable selenium (Köhler et al., 2020). As selenium can bioaccumulate in insects, it seems likely that concentrations are strongly influenced by dietary levels (Arnold et al., 2014).

Since the contents of the gastrointestinal tract can represent a significant percentage of the total weight of the insect (Finke, 2003), it can have a significant effect on the mineral content of the insect if analyzed when fully fed. Additionally, studies on wild-caught insects show both seasonal variations as well as variation between different populations of the same species living in the same general area (Finke, 1984; Studier et al., 1991; Studier and Sevick, 1992).

18.2.6 Vitamins and carotenoids

18.2.6.1 Vitamin A

Vitamin A plays a role in a wide variety of physiological processes including vision, cell differentiation, immune response, reproduction and growth. There are limited data regarding the vitamin A content of wild-caught insects and most captive-bred insects contain relatively low levels of vitamin A/retinol (typically less than 300 μg retinol/kg dry matter) (Barker et al., 1998; Bawa et al., 2020b; Finke, 2002, 2013; Hatt et al., 2003; Oonincx and van der Poel, 2011; Parker et al., 2020; Pennino et al., 1991; Punzo, 2003). Migratory locusts (*Locusta migratoria* L.) fed a grass diet supplemented with wheat bran and fresh carrots contained significantly more retinol than those fed only a grass diet (Oonincx and van der Poel, 2011). However, the retinol levels for all locusts were well below the requirements of the rat.

In fruit flies only the eyes contained measurable quantities of retinoids and the amount detected was a function of the carotenoid content of the larval diet (Giovannucci, Stephenson, 1987; Goldsmith and Warner, 1964; Seki et al., 1998; Von Lintig, 2012). The low values reported in the literature for the vitamin A content of captive-bred insects may be a result of several factors. First, most analytical methods used for vitamin A analysis are specific to retinol and may not detect other retinoids (retinal and 3-hydroxyretinal) found in insect eyes (Goldsmith and Warner, 1964; Seki et al., 1998; Smith and Goldsmith, 1990). It is unclear if 3-hydroxyretinal can serve as a source of vitamin A since it is unknown if 3-hydroxyretinal can be converted into retinal by insectivores. Second, retinoid levels in fruit fly eyes are a function of dietary carotenoid content and diets fed to commercially raised insects may not contain sufficient levels or the correct types of carotenoids to optimize retinal and 3-hydroxyretinal content of insects. Third, many insect species raised for food are fed to insectivores as larvae, which do not possess compound eyes where retinoid synthesis from carotenoids takes place (Von Lintig, 2012).

In addition to retinol, certain carotenoids can be converted into vitamin A in many animal species (Bender, 2002; Levi et al., 2012; McComb, 2010; Olson, 1989). Although it is unclear if all insectivores have the ability to convert beta-carotene and other pro-vitamin A carotenoids to retinol, both leopard geckos (*Eublepharis macularius* Blyth) (Cojean et al., 2018) and Mississippi gopher frogs (*Rana sevosa* Goin and Netting) (Ploog et al., 2015) seem to be able to convert beta-carotene to retinol. Most captive-bred insects contain little if any carotenoids although in a few instances moderately high levels have been reported (Finke, 2002, 2013, 2015a; Jones et al., 1972; Oonincx and van der Poel, 2011; Oonincx and Dierenfeld, 2012). In contrast, high levels of carotenoids are found in wild-caught insects (Arnold et al., 2010; Cerda et al., 2001; Eeva et al., 2010; Helmer et al., 2015; Isaksson and Andersson, 2007; Maoka et al., 2020, 2021; Newbrey et al., 2013; Ssepuuya et al., 2017). The reason for this discrepancy is likely a function of dietary carotenoid intake, as was shown in both fruit flies (Giovannucci and Stephenson, 1999) and silkworm larvae (Chieco et al., 2019). Wild insectivores likely use a combination of retinoids (retinal and possibly 3-hydroxyretinal) and provitamin A carotenoids to meet their vitamin A requirements. A better understanding of the retinoid content of insects and the potential conversion of various retinoids and carotenoids to vitamin A by insectivores is important since vitamin A deficiency has been reported in captive insectivores (Ferguson et al., 1996; Hoby et al., 2010; Miller et al., 2001; Pessier et al., 2005; Wiggans et al., 2018).

18.2.6.2 Vitamin D

Vitamin D_3 is a conditionally essential nutrient for the majority of insectivores as most animal species can synthesize it de novo provided, they are exposed to adequate amounts of UVb radiation (Bos, et al., 2018; Diehl et al., 2018; Oonincx et al., 2018, 2020; Schutkowski et al., 2013; Watson et al., 2014). As most commercially produced insects lack access to UVb, their vitamin D content depends on dietary intake. None of the five commercially produced insect species reached the threshold for detection of 250 IU/kg fresh matter (~595−1445 IU/kg dry matter) (Finke, 2002). Using a more sensitive technique, black soldier fly larvae, butter worm larvae and Turkestan roaches were shown to contain 388−633 IU vitamin D_3/kg dry matter, while adult houseflies were still below the limit of detection (Finke, 2013). A further study reports a low vitamin D_3 level in commercially produced yellow mealworms (150 IU/kg dry matter), but a higher concentration in house crickets (934 IU/kg dry matter) (Oonincx et al., 2010). These highly variable concentrations were attained via dietary accumulation. In wild-caught specimens with the opportunity to expose themselves to UVb, vitamin D_3 levels are highly variable. For instance, wild-caught ant eggs and adult rhinoceros beetles can contain high levels (1288−2806 IU/kg dry matter), whereas these seem lower in pallid-winged grasshoppers (284 IU/kg dry matter) and far lower (<187 IU vitamin D_3) for white-lined sphinx moths (Finke, 2015b; Melo-Ruiz et al., 2013). While further studies are needed to provide insight into the mechanisms behind these variable concentrations, access to UVb plays a role. Providing UVb access to four insect species showed increases in vitamin D_3 concentrations in house crickets (121−361 IU/kg dry matter), migratory locusts (0−557 IU/kg dry matter) and yellow mealworms (163−6973 IU/kg dry matter) as a result of UVb exposure (Oonincx et al., 2018). Conversely, this effect was absent in black soldier fly larvae. Hence, it seems that UVb exposure can make certain feeder insects a significant dietary source of vitamin D_3, although not for others. However, it seems plausible that providing UVb to the insectivores themselves may be a suitable way to ensure an adequate vitamin D status.

18.2.6.3 Vitamin E

Vitamin E serves as an antioxidant and therefore helps maintain the functionality of a variety of lipid-soluble compounds in the body. Insects contain varying amounts of vitamin E. House crickets, yellow mealworms and black soldier fly larvae can contain widely varying levels of vitamin E. Values for house crickets range from 5−131 mg vitamin E/kg dry matter (Barker et al., 1998; Finke, 2002, 2015a; Hatt et al., 2003; Pennino et al., 1991) yellow mealworm larvae range from less than 15 mg/kg to 78 mg vitamin E/kg dry matter (Barker et al., 1998; Finke, 2002, 2015a; Pennino et al., 1991) and black soldier fly larvae range from 6.7 to 104 mg vitamin E/kg dry matter (Finke, 2013; Liland et al., 2017; Liu et al., 2017; Shumo et al., 2019). The large variations appear to be a function of the insect's diet, representing both the vitamin E incorporated into the body tissue as well as the vitamin from the diet remaining in the insect's gastrointestinal tract. The vitamin E content of other commercially raised feeder insects is relatively low (typically less than 15 mg/kg dry matter) (Barker et al., 1998; Finke, 2002, 2013; Jones et al., 1972; Oonincx and Dierenfeld, 2012; Punzo, 2003). Several species, including butterworm larvae and silkworm larvae have somewhat higher levels (33−35 mg vitamin E/kg dry matter) while much higher levels (110−120 mg vitamin E/kg dry matter) are reported for fruit flies, house flies and false katydids (*Microcentrum rhombifolium* Saussure) (Finke, 2002, 2013; Oonincx and Dierenfeld, 2012). Wild-caught insects appear to contain vitamin E at levels on the higher end of the range or exceeding those seen in captive-bred insects (Arnold et al., 2010; Cerda et al., 2001; Finke, 2015b; Fogang et al., 2017; Köhler et al., 2020; Koštál et al., 2013; Pennino et al., 1991; Punzo, 2003; Teffo et al., 2007).

18.2.6.4 B-vitamins

While there is limited comprehensive information regarding the B-vitamin content of most insects, there are reports on the B-vitamin content of the most commonly produced feeder insects (Bawa et al., 2020a; Finke, 2002, 2013, 2015a; Jones et al., 1972). As data on B-vitamin content of unprocessed, wild-caught insects is lacking, it is unclear whether captive-bred and wild-caught insects have similar levels. Furthermore, differences in analytical methods (microbiological vs chemical techniques) and in methods for preparing samples further complicate direct comparisons. Some B-vitamins are relatively unstable when exposed to heat, light or oxygen and can be lost during sample preparation. Therefore, data from dried insects processed for human consumption may underestimate the concentrations in live whole insects. For the same reason, commercially available dried insects may contain lower levels of some B-vitamins due to processing, drying and storage. The data that is available is summarized below.

Thiamine (vitamin B_1) is needed as a cofactor for several important enzymes associated with energy metabolism (Thurnham et al., 2000). A number of species of feeder insects contain fairly low levels of thiamine including house crickets (<0.2−0.7 mg thiamine/kg dry matter), adult yellow mealworms (1.7 mg thiamine/kg dry matter), superworms

(1.4−4.6 mg thiamine/kg dry matter), butterworms (1.8 mg thiamine/kg dry matter), and Turkestan roaches (2.9 mg thiamine/kg dry matter) (Bawa et al., 2020b; Finke, 2002, 2013, 2015a). Other species of feeder insects including black soldier fly larvae (19.8 mg/kg dry matter), adult house flies (44.8 mg thiamine/kg dry matter), silkworms (19.1 mg thiamine/kg dry matter), yellow mealworm larvae (3.5−6.3 mg thiamine/kg dry matter), and waxworms (3.3−5.5 mg thiamine/kg dry matter) contain higher levels (Finke, 2002, 2013, 2015a). A wide range of thiamine levels (0.7−14.3 mg thiamine/kg dry matter) has also been reported for wild-caught rhinoceros beetles, pallid-winged grasshoppers and adult white-lined sphinx moths (Finke, 2015b). Similarly, large variations (0.3−32.4 mg thiamine/kg dry matter) are reported for oven-dried wild-caught Nigerian insects comprising five orders (Banjo et al., 2006). Based on a microbiological method, high thiamine levels (30.2 and 36.7 mg thiamine/kg dry matter) are reported for African palm weevil larva [*Rhynchophorus phoenicis* (F.)] and larvae of the cavorting emperor moth (*Usta terpsichore* (Maassen and Weymer)), while the values for termites [*Macrotermes subhyalinus* (Rambur)] were far lower (1.3 mg thiamine/kg dry matter) (Santos Oliveira et al., 1976). Similarly low levels of thiamine (1.5−2.7 mg thiamine/kg dry product) are reported based on a microbiological method for dried and smoked Attacidae caterpillars from Zaire (Kodondi et al., 1987). As thiamine is relatively unstable and dried insects were processed using a variety of traditional methods (drying, smoking, and frying), unprocessed insects might have higher concentrations (Banjo et al., 2006; Kodondi et al., 1987; Santos Oliveira et al., 1976; Teffo et al., 2007).

Riboflavin (vitamin B_2) is a co-enzyme in the metabolism of a variety of nutrients (Thurnham et al., 2000). Most species of commercially raised feeder insects contain relatively high levels of riboflavin ranging from 17.6 to 306.3 mg/kg dry matter (Bawa et al., 2020b; Finke, 2002, 2013, 2015a; Jones et al., 1972). Similar levels (32.4−39.2 mg riboflavin/kg dry matter) were found for honeybee brood (larvae and pupae) (Banjo et al., 2006; Finke, 2005) and wild-caught rhinoceros beetles, pallid-winged grasshoppers and adult white-lined sphinx moths (66.5−82.7 mg riboflavin/kg dry matter) (Finke, 2015b). Dried and smoked Attacidae caterpillars also contain high levels of riboflavin (32−51 mg/kg dry product) (Kodondi et al., 1987). These values are higher than those for termites, palm weevil larvae and a saturnid caterpillar processed for human consumption (9.0−22.4 mg riboflavin/kg dry matter) (Santos Oliveira et al., 1976; Teffo et al., 2007). Banjo reports highly variable levels of riboflavin in 14 species of insects from Nigeria ranging from 0.9 to 32.4 mg/kg dry matter (Banjo et al., 2006). After collection, these samples were kept dry (adults) or stored in 70% alcohol (larvae). Since riboflavin is degraded by light it is unclear if these represent real differences or are a result of losses during storage prior to analysis.

Niacin (vitamin B_3) plays a role in metabolism and tissue respiration (Thurnham et al., 2000). It appears abundant in commercially raised feeder insects, with levels ranging from 77 to 359 mg/kg dry matter (Finke, 2002, 2013, 2015a). Bawa et al. (2020b) obtained much lower values (3.6−30.1 mg niacin/kg dry matter) for house crickets compared to previous studies (107−143 mg niacin/kg dry matter) (Finke, 2002, 2015a). The reason for these discrepancies is unknown but may be related to analytical methods. Wild-caught rhinoceros beetles, pallid-winged grasshoppers and adult white-lined sphinx moths also contained high levels of niacin (96.4−191.4 mg niacin/kg dry matter) (Finke, 2015b). A variety of dried insects consumed in Africa, including termites, palm weevil larvae and several species of caterpillars, have been analyzed for niacin. Even though these were processed with a variety of methods (drying, smoking and frying) all had high levels of niacin, ranging from 52 to 110 mg/kg dry matter (Kodondi et al., 1987). Conversely, larvae of the cavorting emperor moth contain only 3 mg niacin/kg dry matter (Santos Oliveira et al., 1976). As niacin is a relatively stable B-vitamin these values are likely representative of live insects.

Pantothenic acid (vitamin B_5) functions as a component of coenzyme A and as such plays a role in the citric acid cycle, fatty acid synthesis and oxidation reactions. It is widely distributed in most foodstuffs and commercially raised insects appear to be no exception, with levels ranging from 18.9 to 179.8 mg/kg dry matter (Finke, 2002, 2013, 2015a). Bee brood, wild-caught rhinoceros beetles, pallid-winged grasshoppers, and adult white-lined sphinx moths also appear to be good sources of pantothenic acid (41.8 to 56.5 mg pantothenic acid/kg dry matter) (Finke, 2005, 2015b). There are little additional data available on the pantothenic acid content of wild-caught insects although using a microbiological assay dried smoked Attacidae caterpillars were found to contain very low levels of pantothenic acid (0.073−0.102 mg/kg dry product) (Kodondi et al., 1987).

Pyridoxine (vitamin B_6) plays an important role in several metabolic reactions including amino acid metabolism. Commercially raised insects appear to be good sources of pyridoxine with values ranging from 1.3 to 4.8 mg pyridoxine/kg dry matter for waxworm larvae to 22.3 mg /kg dry matter for yellow mealworm beetles (Bawa et al., 2020b; Finke, 2002, 2013, 2015a; Jones et al., 1972). Most insect species, including three species of wild-caught insects, contain between 5 and 10 mg pyridoxine/kg dry matter. Very low levels of pyridoxine (0.37 to 0.63 mg/kg) were detected in three species of dried smoked Attacidae caterpillars using a microbiological technique (Kodondi et al., 1987). These low values might be due to the smoking and drying processes used to preserve these insect products as pyridoxine is susceptible to degradation by heat.

Biotin (vitamin B_8) carries carboxyl groups in ATP reactions. The biotin content of both commercially raised and wild-caught insects is highly variable ranging from 0.17 to 2.69 mg/kg dry matter, however, the values for most species ranged from 0.7 to 1.4 mg/kg dry matter (Finke, 2002, 2013, 2015a). Dried smoked meal from Attacidae caterpillars has been reported to contain 0.23–0.45 mg/kg (Kodondi et al., 1987). It is unclear if these low values reflect biological differences, biotin loss during processing (drying and smoking), or analytical techniques used.

Folic acid (vitamin B_9) plays an essential role in DNA synthesis and one carbon metabolism (Thurnham et al., 2000). Using a chemical method, commercially raised insects were shown to contain high levels of folic acid ranging from a low of 1.38 mg/kg for waxworm larvae to 7.22 mg/kg for house flies (Finke, 2002, 2013, 2015a). Lower values were observed for three species of wild-caught insects (0.17 to 2.4 mg folic acid/kg dry matter). In contrast, very low folic acid values (0.20–0.63 mg folic acid/kg dry matter) were found for smoked and dried product from three species of Attacidae caterpillars using a microbiological method (Kodondi et al., 1987). Since folic acid is susceptible to degradation both by light and oxidizing agents the low values may be due to the smoking and drying process used to preserve the insect product.

Cobalamin (vitamin B_{12}) is found exclusively in animal products and plays a key role in reactions involving methyl donors and concentrations in insects vary greatly. At the lower end, are commercially raised silkworm larvae, waxworm larvae and wild-caught adult sphinx moths, with levels below 3–4 μg/kg dry matter (limit of detection) (Finke, 2002, 2015b). These are followed by low levels in yellow mealworm larvae and beetles, as well as superworms (1.3–15 μg/kg/dry matter; Finke, 2002, 2015a; Lenaerts et al., 2018; Schmidt et al., 2019). Levels in Jamaican field crickets are somewhat higher (28.8 μg/kg dry matter; Schmidt et al., 2019), but not as high as most house crickets (174–702 μg/kg dry matter; Finke, 2002, 2015a). Although data is limited, it seems that black soldier fly larvae (144 μg/kg dry matter; Finke, 2013) and Turkestan cockroaches (132–767 μg/kg dry matter) are both good sources of vitamin B_{12} (Finke, 2013; Schmidt et al., 2019). Similarly, high vitamin B_{12} levels (140–250 μg/kg dry matter) are reported for dried and smoked products from three species of Attacidae caterpillars (Kodondi et al., 1987).

18.2.6.5 Vitamin C

Vitamin C is needed to form connective tissue and functions as an antioxidant. Insects contain some vitamin C and while some are able to synthesize it de novo, others depend on nutritional sources (Kramer and Seib, 1982). Honeybees contain relatively high amounts of vitamin C (103 to 164 mg/kg dry matter) (Banjo et al., 2006). Whereas both adult house crickets and adult mealworms contain similar levels (97–149 mg/kg dry matter), most other species contain less than 50 mg/kg dry matter (Finke, 2002, 2007, 2013; Banjo et al., 2006).

18.2.7 Other nutrients

In addition to the aforementioned nutrients, insects contain a variety of other compounds that may be important to help maintain the health of insectivores. These include choline, taurine, and various sterols including cholesterol (Finke, 2015a, 2015b; Cerda et al., 2001; Ramsay and Houston, 2003).

18.2.7.1 Choline

Choline is a component of both lecithin and the neurotransmitter acetylcholine and also plays an important role in one-carbon metabolism (Pesti et al., 1979). As such it can spare the need for dietary methionine an important consideration given that methionine is typically the first-limiting amino acid in most insect species as was noted earlier. While most reported choline analysis is from commercially raised feeder insects, the data shows insects contain high levels of choline (1570–7258 mg/kg dry matter) (Finke, 2002, 2013, 2015a, 2015b; Fogang et al., 2017; Noland and Baumann, 1949).

18.2.7.2 Taurine

Taurine, an amino sulfonic acid, is involved in cell volume regulation, provides a substrate for the formation of bile salts, and plays a role in the regulation of intracellular calcium. It is a required nutrient for some species, such as cats and foxes (Hayes et al., 1975; Moise et al., 1991), but most species can synthesize taurine from methionine provided sufficient precursors are available. The only insectivore studied, the giant anteater (*Myrmecophaga tridactyla* L.) has been shown to synthesize taurine from dietary methionine (Nofs et al., 2018). House crickets, grasshoppers, adult fruit flies, house flies, and moths are rich sources of taurine containing (2800–7300 mg/kg). In contrast adult beetles and most species of insect larvae contain little if any taurine (Bodnaryk, 1981; Finke, 2002, 2013, 2015a, 2015b; Giannetto et al., 2020b; Massie et al., 1989; Ramsay and Houston, 2003). The highest levels of taurine in insects are found in the flight muscles which is likely why most adult holometabolous insects contain high levels of taurine (Whitton et al., 1987). It has been proposed that adult passerines have a preference for spiders (7200−21,350 mg taurine/kg dry matter), driven by a need for enhanced levels of methionine, cystine, or taurine by their growing chicks (Ramsay and Houston, 2003). Supplementing the diet of wild blue tit (*Cyanistes caeruleus* L.) nestlings from 2 to 12 days of age with taurine resulted in juveniles that took significantly greater risks when investigating novel objects and were more successful at a spatial learning task than controls (Arnold et al., 2007). The significance of dietary taurine for other species of insectivores is unknown.

18.2.7.3 Sterols

Sterols play a critical role as structural components in the phospholipid bilayer of cell membranes. The dominant sterols in most insects are β-sitosterol, 7-dehydrocholesterol and cholesterol, although insects contain a variety of other sterols including campesterol and stigmasterol (Cerda et al., 2001; Connor et al., 2006; Jing and Behmer, 2020; Koštál et al., 2013; Liland et al., 2017; Mlček et al., 2019; Sabolová et al., 2016; Svoboda et al., 1995). Both the total sterol content and the relative amounts of the various sterols appear to be a function of species, diet, life stage and season (Connor et al., 2006; Koštál et al., 2013; Liland et al., 2017). For instance, in two species of sawflies (Hymenoptera) the predominant sterol was cholesterol (57%−73%), whereas in a third species 75% of the sterols present were in the form of 7-dehydrocholesterol (Svoboda et al., 1995). This indicates that large differences can exist in the primary storage form, even in closely related species. In contrast, in four cricket species provided with the same diet, the predominant sterol was cholesterol (83%−92%) at a concentration of 10−15 mg/g of fat (Tzompa-Sosa et al., 2021). High dietary levels of cholesterol increase the risk of atherosclerosis in humans and some monkey species, but the significance of dietary sterols for most insectivores is unclear. Corneal lipidosis and hypercholesterolemia has been reported in Cuban Tree Frogs (*Osteopilus septentrionalis* Duméril and Bibron) fed a diet of house crickets and occasionally day-old mice (Russell et al., 1990). In a later study Cuban tree frogs fed house cricket containing extremely high levels of cholesterol (1.7% dry matter basis) had a much higher incidence of corneal lipidosis than those fed house crickets containing 0.7% cholesterol or wild-caught Cuban tree frogs (Shilton et al., 2001).

18.3 Effects of insect size/life stage on nutrient composition

In general, the lipid content of wild-caught insects is approximately 30% for larvae and 20% for adults (Fast, 1970). Fat stores are usually greatest in the final larval stage prior to metamorphosis (Fast, 1970). This is more apparent for species which undergo complete metamorphosis (holometabolous species; for instance, yellow mealworms) than those with incomplete metamorphosis (hemimetabolous species; for instance, house crickets).

Hence, larvae of holometabolous species have a higher fat content than adults (Cookman et al., 1984; Finke, 2002; Lease and Wolf, 2011; Punzo, 2003). Yellow mealworms, for example, accumulate fat during larval growth (Finkel, 1948; McClements et al., 2003). These reserves are used as an energy source during metamorphosis, resulting in a lower fat content for adults and a thereby an increase in the relative content of protein and ash (Barker et al., 1998; Bernard and Allen, 1997; Downer and Matthews, 1976; Finke, 2002; Lease and Wolf, 2011; Oonincx and Dierenfeld, 2012; Ravzanaadii et al., 2012). A similar trend is seen in fruit flies (Church and Robertson, 1966), house flies (Pearincott, 1960) and black soldier flies (Liu et al., 2017). Furthermore, during metamorphosis the protein composition of yellow mealworms change; glycine, tryptophan and taurine content increase, while tyrosine content decreases (Finke, 2002; Ravzanaadii et al., 2012). The taurine content of holometabolous fruit flies (Massie et al., 1989), bertha armyworms (*Mamestra configurata*) (Bodnaryk, 1981) and yellow mealworms (Finke, 2002), increases after metamorphosis. This is consistent with the high concentration of taurine found in insect flight muscles (Whitton et al., 1987). The fly *Agria affinis* (Fallen) uses two-thirds of its fat reserve during pupation. This coincides with a relative decrease in C16:0, C16:1 and C18:1, and a relative increase in C18:2 and C22:1 (Barlow, 1965). Similarly, the fat content and fatty acid profile of black soldier fly larvae changes during development. Fat accumulates during the larval stage and then declines during pupation. Both C12:0 and C14:0 increase during larval growth while 16:0 and C18:1 decline (Liu et al., 2017; Giannetto et al., 2020a). Different changes in fat composition occur in silkworms. During their larval development, both fat content and composition change; the total fat content increases with relative increases of C16:0, C16:1, and C18:1 and decreases of C18:0 and C18:2. During pupation, C18:1 further increases while C16:0 continues to decrease. In the velvet bean caterpillar, total lipid content decreases during metamorphosis however C16:0 and C18:1 levels increase, while C18:3 levels decrease (Cookman et al., 1984). To what extent these changes are species-specific or constitute differences between Diptera and Lepidoptera needs further investigation. Similar to species preparing for pupation, preparation for diapause or hibernation results in increased energy (fat) reserves (Ali and Ewiess, 1977; Downer and Matthews, 1976). In aquatic insects the concentration of C20:4 (arachidonic acid) and C20:5 decrease after the larval phase, when the insects leave the aquatic environment (Hanson et al., 1985; Sushchik et al., 2013).

Unlike holometabolous insects, hemimetabolous adults tend to have a higher fat content than nymphs (Lease and Wolf, 2011; Lipsitz and McFarlane, 1971). This holds true for some but not all studies conducted on migratory locusts (Oonincx et al., 2010; Oonincx and van der Poel, 2011). Probably the time of sampling, whether directly after adult emergence or later during adulthood, explains these differences since fat continues to accumulate after adult emergence (Beenakkers et al., 1985). Generally, flying and migratory insects accumulate more fat, which is used as an energy source for flight (Downer and Matthews, 1976). In this locust species, the dry matter content increases between the penultimate instar and adulthood (Oonincx and van der Poel, 2011). Interestingly, in both migratory and desert locusts (*Schistocerca gregaria* Forsskål) C18:3n3 comprises 9%−12% of the fatty acids in penultimate specimens, whereas this fatty acid is absent in adults (Oonincx et al., 2015). Similar to migratory locusts, the dry matter content of house crickets significantly increases (from 23% to 30%) during the penultimate stage of development (Roe et al., 1980). Adult house crickets have a higher protein and a lower fat content than small and medium nymphs (Finke, 2002; McClements et al., 2003). The amino acid composition seems to remain similar between nymphs and adults of this species (Finke, 2002). In a study of three cockroach species [Turkestan cockroaches, six-spotted cockroaches (*Eublaberus distanti* (Kirby)) and hissing cockroaches (*Gromphadorhina portentosa* (Schaum))], an increase in dry matter and crude protein content, and a concomitant decrease in fat content, was noted between small and medium specimens. The opposite change was noted when six-spotted roaches reached adulthood (Oonincx and Dierenfeld, 2012).

Besides size differences, certain gender differences seem apparent in insects, males often having a lower body weight than females, which might be caused by a lower fat content as mentioned previously (Ali and Ewiess, 1977; Hoffmann, 1973; Sonmez and Gulel, 2008).

18.4 Effects of insect diet on insect nutrient composition

Diet can have a significant effect on the nutrient composition of an insect. Since the entire insect is normally consumed, dietary effects described in the literature can partially be due to diet remaining in the gut which is discussed in more detail later in this chapter. The body composition of the insect itself can also be altered to a certain extent through the diet. For instance, higher water content of wheat bran (8.7 vs 6.7%) provided to yellow mealworm larvae increases their water content (64 vs 59%) (Machin, 1975).

For the macro nutrients, it seems that insect fat content is highly variable and that the relative content of protein and ash are subsequently affected. If an insect with a certain amount of protein increases its fat reserve, the percentage of protein thereby decreases (Oonincx and van der Poel, 2011; Simpson and Raubenheimer, 2001; St-Hilaire et al., 2007). This might be the reason why the protein content stays constant during adulthood, if a protein source is available and protein content decreases if only a carbohydrate source, which can be converted to fat, is available as was shown in Mediterranean fruit flies [*Ceratitis capitata* (Wiedemann)](Nestel et al., 2005). Although the percentage of protein can be altered through the diet, it seems unlikely that the amino acid composition is affected as mentioned previously.

As stated before, the crude fat content of insects can be altered by the diet provided. Two independent studies on fruit flies using the same artificial diet showed a similar nutrient composition. A third study on fruit flies found dissimilarities in fat and iron, which could indicate a strong dietary influence (Barker et al., 1998; Bernard and Allen, 1997; Oonincx and Dierenfeld, 2012). For house crickets, several studies are available on their chemical composition. Large differences in fat content have been reported ranging from 17% to 37% dry matter (Barker et al., 1998; Finke, 2002; Hatt et al., 2003; Oonincx et al., 2010). It seems likely that variation in fat content is a result of both the diet provided and the age of the cricket (Hatt et al., 2003). The protein and fat content of adult mealworm beetles can also be significantly affected by the protein and carbohydrate content of the larval diet (Rho and Lee, 2014).

Besides the plasticity of total fat content, large differences can be expected in the fatty acid composition when different diets are provided. In nonruminant production animals, short term provision of poly-unsaturated fatty acids increases the poly-unsaturated fatty acid content of the meat indicating that these fatty acids were incorporated in the body (Kouba and Mourot, 2011). Studies on insects indicate that the fatty acid composition of both larvae and adults tends to reflect the fatty acid composition of the diet provided (Cookman et al., 1984; Madariaga et al., 1971; Meneguz et al., 2018; Schaefer, 1968; St-Hilaire et al., 2007; Starčević et al., 2017; Rutaroa et al., 2018; van Broekhoven et al., 2015). The fatty acid composition of the insect however does not directly match that of the diet, suggesting selective accumulation or synthesis (Cookman et al., 1984; Hoc et al., 2020). For instance, in migratory locusts, the C18:1 and C18:3 content of the diet strongly relates to the fatty acid content of the locust fat body. However, for C18:2 this was not the case, possibly due to poor absorption or due to conversion to C18:1 via saturation (Beenakkers and Scheres, 1971). While diet composition influences the fatty acid composition directly, indirect effects have also been reported. For instance a sufficient supply of C18:2, can be used to synthesize C20:4 and C20:5 (Hanson et al., 1983). Another prime example is the de novo synthesis of C12:0 in black soldier fly larvae (Hoc et al., 2020). Whereas C12:0 is the predominant fatty acid in this species, the total fatty profile can be greatly affected, for instance by supplying (C18:3n3) (Oonincx et al., 2019).

For many feeder insects, carrots are a well-accepted part of the diet or a means of providing moisture. In migratory locusts, the addition of carrots increases carotene and retinol (vitamin A) content (Oonincx and van der Poel, 2011). While the elevated carotene levels could be due to gut loading, it seems likely that the increased retinol content is due to the conversion of carotene to retinol. Likewise, when the medium of fruit fly larvae was enriched with carrot juice, larva, pupa and adults contained enhanced levels of both β-carotene and xanthophylls although the relative amounts varied between larva and adults (Giovannucci and Stephenson, 1999). Fruit flies can convert β-carotene to retinal, suggesting that more insect species have this ability (Von Lintig and Vogt, 2000). Great tits (*Parus major* L.) are able to distinguish carotenoid-enriched yellow mealworms from nonenriched counterparts and prefer the former (Senar et al., 2010).

18.5 Effects of environment on insect composition

A number of environmental factors, such as temperature, light and humidity, can affect growth, development and chemical composition of insects.

18.5.1 Temperature

Insects are poikilothermic, that is, cold-blooded; their body temperature depends to a large degree on the environmental temperature. Therefore within a range of temperatures suitable for the specific species, their metabolic rate and growth rate increases with higher temperatures (Akman Gündüüz and Gülel, 2002; Ali and Ewiess, 1977; Ali et al., 2011; Angilletta et al., 2004; Krengel et al., 2012). A standard way of quantifying this is the relative difference when the temperature is increased by 10 degrees (Q10). For instance, in house crickets the development time is halved with a 10-degree increase in temperature (Roe et al., 1980). While growth rates are increased by higher temperatures, adult size generally seems to decrease (Akman Guünduüz and Gülel, 2002; Angilletta et al., 2004; Krengel et al., 2012) although the opposite effect was observed for both desert locusts and migratory locusts (Akman Guünduüz and Gülel, 2002; Beenakkers et al., 1971). Last instar female house crickets gain weight more quickly and more efficiently at 35°C than at 25°C, or 30°C (Roe et al., 1980, 1985). At higher temperatures, lipid content decreases during the latter half of this instar, but at 25°C carbohydrates are still converted to lipids. It seems that optimal growth occurs at 35°C, which is similar to the 34°C for the two-spotted field cricket (Hoffmann, 1973). However, for the latter species, mortality is also the highest at 34°C. Both growth rate and mortality are decreased at 27°C. For two-spotted field crickets temperature changes during rearing (alterations between 20°C and −1.5°C) versus constant temperatures (13°C) increase water content and decrease protein content (Hoffmann, 1973). Alternating temperatures around the optimal growth temperatures appear to increase protein and fat content in this species (Hoffmann, 1973). At low temperatures (13° and 20°C vs 27°C), fat content is higher, as are the proportions of unsaturated fatty acids. This seems a likely adaptation to the thermal regime. In the fly *Pseudosarcophaga affinis* (Fallén), higher proportions of saturated fatty acids increase heat tolerance (House et al., 1958). A comparative study on the seven-spotted [*Coccinella septempuctata* (L.)] and the Asian lady beetle [*Harmonia axyridis* (Pallas)] indicated that the first species accumulates more fat at elevated temperatures compared to normal temperatures (18° vs 21°C). Fat accumulation remains low under both conditions in the latter species. Females of the seven-spotted lady beetle have a higher fat content than males at normal temperatures, but a similar fat content at elevated temperatures. The carbohydrate and protein content of bean beetles [*Acanthoscelides obtectus* (Say)] is lower at 20°C than at 30°C, but lipid content is the same (Sonmez and Gulel, 2008).

18.5.2 Humidity

Humidity is normally expressed as relative humidity (RH), which is the relative amount of water that can be stored in the air at a certain temperature. Higher levels of humidity, within an appropriate range, seem to allow for more rapid development (Ali et al., 2011; Han et al., 2008; Nedvěd and Kalushkov, 2012). However, unlike in the case of increasing temperatures, this increase in growth rate does not seem to lead to a lower body mass in adults. For instance, the body mass of the pine caterpillar, *Dendrolimus tabulaeformis* (Tsai et Liu) increases with higher humidity during their larval stages (20%–100%) (Han et al., 2008).

A comparative study on the effects of humidity and temperature on locust species (*L. migratoria*, and *S. gregaria*) indicated development is possible between 35% and 80% RH, with 60%–65% being optimal, and increasing temperatures require a higher RH (Hamilton, 1936). Similarly, pine caterpillars require an RH above 40% for development, while the optimal RH for maximal body mass seems to be around 80%. During diapause, this species absorbs water from the substrate, thereby increasing its live weight (Han et al., 2008). Fasting mealworms are capable of absorbing water vapor if RH is above 88% RH (Fraenkel, 1950; Machin, 1975). RH also has an indirect effect through the feed provided as well. If the feed provided to yellow mealworms is in equilibrium with 70% RH, it allows for rapid growth (Fraenkel, 1950). High humidity increases the dry matter weight gain of yellow mealworms postexposure (Machin, 1975). However long-term exposure to high humidity (> 85%) results in higher larval mortality, probably due to excessive hydration (Machin, 1975). Other problems are likely to occur at high RH, for instance, the development of fungi and/or mites (Machin, 1975). The optimal growth rate for mealworms at 25°C is attained at 70% RH (Fraenkel, 1950; Machin, 1975).

As indicated before, the optimal RH also relates to temperature, although other variables can play a role as well. The Asian lady beetle tends to grow larger at higher humidities (between 30% and 90%) if fed with the aphid *Acyrthosiphon pisum* (Harris) but not when fed on frozen eggs of *Ephestia kuehniella* (Zeller) (Nedvěd and Kalushkov, 2012).

It seems that the body weight and development rate of insects are higher at the top of their RH range. The moisture content of insects also seems to increase at a higher RH. However, little is known about how body composition is affected by changes in RH.

18.5.3 Photoperiod

The effect of photoperiod (daily exposure time to light) on insect composition has not been studied in detail; most studies have focused on behavioral effects, fecundity and body weight. For certain locust species, long photoperiods (up to 24 hours) increase their growth rate, possibly as a result of increased food intake. At a constant temperature, Asian lady beetles tend to develop more quickly with 16 hours of light compared to 12 hours of light, but the adult weight was similar (Berkvens et al., 2008). This seems likely for these diurnal species, but the green stink bug [*Nezara viridula* (L.)] that mainly feeds at night, has an increase in the rate of development with an increased photoperiod (10 vs 14 hours) resulting in an increase in body weight when reaching, and during adulthood (Ali and Ewiess, 1977; Shearer and Jones, 1996). Short photoperiods can induce diapause, while longer photoperiods are more likely to induce reproduction. It seems that for this species, more energy is accumulated when preparing for reproduction than for diapause, which would explain the higher fat content. However, little is known about the influence of photoperiod on the chemical composition. The protein content of adults of the giant wax moth increases more rapidly if kept in constant light, compared to constant darkness (Koc and Gulel, 2008). The effects of photoperiod on the nutrient profile of insects mostly seem to be indirect, acting through other processes, such as preparation for reproduction or diapause or concomitant changes in temperature.

18.6 Nutrient requirements of insectivores including nutrient availability

It is difficult to compare the nutritive value of insects as a group to the dietary requirements of insectivores because of the large number of insect species and the large differences in nutrient content between those species (Barker et al., 1998; Bukkens, 1997; Finke, 2002, 2013). A proper evaluation of a food/insect requires it to be evaluated in the broader context of a complete diet, made up of a number of different foods/insects.

18.6.1 Availability and digestibility

It has been suggested that insects might be poorly digestible because of their sclerotized, chitin-rich, exoskeletons. Due to the recent interest in the use of insects as feed ingredients for production animals, there is now an abundance of data on the protein digestibility of various insect meals. Of note, however, is that the insects used in these feeding trials are heated to remove excess moisture, finely ground and in some cases defatted and then incorporated into mixed diets. Hence, the application of these values to that of raw whole insects is unclear. That said, in most studies, the protein and amino acid digestibility of insect meals equals, or surpasses the more conventional protein sources. This includes studies when insects meals are fed to rats (Dreyer and Wehmeyer, 1982; Xia et al., 2012), pigs (Biasato et al., 2019; Jin et al., 2016; Yoo et al., 2018), shrimp (Panini et al., 2017), various species of poultry (Benzertiha et al., 2019; Bovera et al., 2016; De Marco et al., 2015; Hall et al., 2018; Hwangbo et al., 2009; Pieterse and Pretorius, 2013; Schiavone et al., 2017; Wang et al., 2007; Woods et al., 2019), and fish (Alegbeleye et al., 2012; Basto et al., 2020; Belforti et al., 2015; Belghit et al., 2019; Chemello et al., 2020; Dumas et al., 2018; Fontes et al., 2019; Gasco et al., 2016; Magalhaes et al., 2017; Piccolo et al., 2017; Renna et al., 2017).

There are some reports on whole dried insects fed to rats in purified diets. Unlike the feeding trials mentioned previously, in these studies the material was freeze-dried to remove excess moisture and the dried insects were the only source of protein in the diet. These studies showed that the apparent protein digestibility of ground, freeze-dried yellow mealworm larvae was 75%−91% when fed to rats (Goulet et al., 1978; Jensen et al., 2019; Poelaert et al., 2018), while for ground freeze-dried house crickets apparent protein digestibility was 78%. (Poelaert et al., 2018). The digestibility of these insects was only slightly lower than for casein (88%−93%), a highly digestible milk protein (Goulet et al., 1978; Poelaert et al., 2018). Similarly, Jensen et al. (2019) report a high true protein digestibility of freeze-dried yellow mealworms (92%) and lesser mealworms (94%) when fed to rats.

There are two studies regarding the protein digestibility of raw whole insects when fed to insectivores. Crude protein digestibility of black soldier fly larvae fed to mountain chicken frogs [*Leptodactylus fallax* (Müller)] was low (44%) for live larvae, but the digestibility increased to 77% when the larvae were mashed (Dierenfeld and King, 2008). The crude protein digestibility of intact house crickets when fed to these frogs was 95% (Dierenfeld and King, 2008). The lower protein digestibility of intact versus mashed larvae could be due to the protective properties of the mineralized exoskeleton, inhibiting the digestives enzyme's access to the more digestible nutrients. The protein digestibility of black soldier fly larvae in leopard geckos (*Eublepharis macularius*) was 81% (Boykin and Mitchell, 2021). These geckos are more likely than mountain chicken frogs to chew on their prey, thereby mechanically damaging the exoskeleton.

In general, protein digestibility of insects is high and the variability reported in the literature is likely a result of differences in how the insects were prepared and the proportion of amino acids used for sclerotization. Insects with a large proportion of their amino acids in a cuticular protein, complexed with chitin, might have a lower protein digestibility than those that do not (Finke, 2007).

Besides protein digestibility some information on mineral digestibility is available. Concerns have been raised about calcium availability from black soldier fly larvae, especially for insectivores that swallow their prey whole. When fed to mountain chicken frogs the calcium and phosphorus digestibility of whole black soldier fly larvae was only 44% and 51% respectively compared to 88% and 91% for larvae that had been "mashed" (Dierenfeld and King, 2008). For comparison when fed to mountain chicken frogs the calcium and phosphorus digestibility for crickets was 84% and 93%, respectively (Dierenfeld and King, 2008). Similarly, the calcium and phosphorus digestibility of whole and "pierced" black soldier fly larvae when fed to leopard geckos was 43% and 45%, respectively (Boykin et al., 2020). Even with a low availability, the high calcium concentration in these larvae should provide adequate amounts of calcium for most insectivores.

18.7 Enhancing the nutrient composition of insects as food for insectivores

While the exact nutrient requirements for most insectivores are unknown, certain nutrient deficiencies are known to occur regularly in captive insectivores. The three most commonly reported are calcium, vitamins A, and D deficiencies. Insectivore diets can be enhanced by increasing the concentration of nutrients which are expected to be present at too low of a level in the insect. There are three main methods to do so; gut-loading, dusting or feeding the insect an enriched diet during growth. The goal of all these methods is to increase the intake of selected nutrients by the insectivore. While this is valid for a certain range, one must be careful that this range is not surpassed, resulting in adverse effects. For instance, over-supplementation with calcium can decrease the absorption of other minerals, leading to secondary trace mineral deficiencies. Likewise, over-supplementation of the fat-soluble vitamins, in particular vitamins A and D, can cause toxicity (Bender, 2002). A difference in size (developmental stage) of the dusted or gut loaded insect leads to differences in a surface: volume and gut size: volume ratios. Hence, smaller insects have a larger relative surface area to which dust can adhere. The same powder used for pinhead or adult house crickets, therefore, has a greater effect on the smaller specimens (Sullivan et al., 2009). Similarly, for gut-loaded insects size differences could lead to differences in nutrient delivery of smaller versus larger insects (Finke, et al., 2005).

18.7.1 Gut loading

Gut loading is the term used for the provision of a special diet to insects, shortly before the insects are consumed. These diets contain high levels of the desired nutrient(s), which are present in the insect gut when the insect is consumed. Therefore, the insectivore's intake of those nutrients increases. Due to the nature of gut loading, it is suitable for almost all nutrients as long as the diet is palatable to the insect and the diet contains sufficient quantities of the desired nutrient(s) (Hunt-Coslik et al., 2009).

Most research on the effects of gut loading has focused on increasing calcium content. High calcium gut load diets containing 4%−9% calcium, typically from calcium carbonate, are effective in increasing the calcium content of wax moth larvae, house crickets, yellow mealworm larvae, superworm larvae and silkworm larvae (Allen and Oftedal, 1989; Anderson, 2000; Finke, 2003; Finke, et al., 2005; Klasing et al., 2000; Latney et al., 2017; Strzeleqicz et al., 1985). Chemical analysis of the diet is recommended to verify the true calcium content of commercially available gut-loading diets (Finke, et al., 2004, 2005). The calcium from yellow mealworms gut loaded with a high calcium diet was readily available to growing chicks showing the usefulness of this method (Klasing et al., 2000). The optimal gut loading time seems to vary slightly. This is likely a result of the insect species, diet palatability and environmental conditions (temperature, light and humidity). In general, however, gut loading for 24−72 hours appears to result in similar levels of nutrients in the intact insect. When gut-loading diets are fed for longer periods of time adverse effects on the viability of the insects can occur (Klasing et al., 2000). In yellow mealworms a gut loading period of 24 hours increased calcium levels and improved calcium to phosphorus ratios, and extending this period to 48 or 72 hours, resulted in a further increase (Klasing et al., 2000; Anderson, 2000). For house crickets, a period of 48 hours seems sufficient to attain a significant increase in calcium to phosphorus ratios, while extending this to 72 hours does not affect this ratio (Anderson, 2000). However, other studies report the highest calcium content after 1 day compared to 2, 3, or 7 days (Dikeman et al., 2007). Dietary calcium to phosphorus ratio of 1:1 to 2:1 is considered optimal for most species. Offering certain gut loading diets longer than two days reduces the initially increased calcium levels (Hunt-Coslik et al., 2009), which could be an effect of diet palatability (McComb, 2010).

In addition, the physical form of the nutrient and the presence of other nutrients affecting palatability should be considered when designing a gut loading diet (Anderson, 2000; Finke, et al., 2005). The addition of poly-unsaturated fatty acids to the gut loading diet has been suggested for insectivores from temperate climates which would be likely to encounter insects with relatively high poly-unsaturated fatty acids concentrations (Li et al., 2009).

Gut-loading can also increase the vitamin A content of crickets, yellow mealworm larvae, black soldier fly larvae and silkworm larvae (Boykin and Mitchell, 2021; Finke, 2003). Insect retinol levels increased either linearly or curvilinearly with increasing dietary levels and at the highest dietary level insects contained sufficient vitamin A to meet the anticipated requirements of most insectivores. When gut-loading insects with vitamin A it is critical that the physical characteristics of the supplement, such as particle size and hardness, allow for adequate consumption by the insect (Livingston et al., 2014).

A study in which yellow mealworms were gut loaded with chicken starter feed, increased their vitamin D content to 132 IU/kg dry matter, while undetectable levels were present in mealworms provided a wheat bran diet (Klasing et al., 2000). A simple way of providing extra carotenoids to insectivores, is the provision of carrots or other fruits or vegetables during the last 24 hours before feeding the insects to the insectivores. The amount of carotenoids that accumulate differs per insect species. The field cricket, for instance, accumulates more carotenoids on a high carotenoid diet than the house cricket, or the banded cricket [*Gryllodes sigillatus* (Walker)] (Ogilvy et al., 2012).

Gut-loading can also increase the levels of omega-3 fatty acids in black soldier fly larvae (Barroso et al., 2017). Larvae fed a control diet with 40% added fish meal had increased levels of the fatty acids 20:5 and 22:6. The omega-3 fatty acid level in the larvae increased rapidly and appeared to reach a plateau after about 3 hours suggesting the increases were primarily a result of gut-loading and not accumulation in the tissues of the insect.

18.7.2 Dusting

Dusting is a term used for coating an insect with a fine powder containing the desired nutrients, such that the powder adheres to the outside of the insect. When the insect is eaten, this powder is also ingested. Little scientific data exist on the nutritional effects of this method. One study in which house crickets were dusted with calcium carbonate powder, the calcium to phosphorus ratio increased from 1:5.7 to 5.3:1 and the digestibility of these minerals was high (84% and 94%, respectively) (Dierenfeld and King, 2008). Whereas dusting can be an effective method for animals which immediately consume their prey, the effectiveness decreases with the time between dusting and consumption (Trusk and Crissey, 1987). House crickets, for instance, can groom off half of the adhering powder in 150 seconds (Li et al., 2009). Another study, quantifying the amount of calcium carbonate adhering to insects after dusting indicated that house cricket weight increased by 13.4% directly after, whereas this decreased to 8.7% after 15 minutes, and 4.5% after 30 minutes (Oonincx et al., 2020). For two locust species the starting weight increased by 5.5%−5.6% due to dusting and halved after 15 minutes, and then again halved after 30 minutes. Another downside of dusting is that it is difficult to quantify the amount of dust adhering to the insect, and hence the dosage provided to the insectivore. This would depend on the physical characteristics of both the dust and the insect exoskeleton, and the relative surface area of the insect. The weight of giant wax moth larvae, yellow mealworm larvae and house crickets increased by 0.8%−6.3% when dusted with two types of calcium carbonate dusts (Winn et al., 2003). For aquatic insectivores, this way of enhancing the nutrient content is obviously unsuitable. Both the composition and the method of providing the dust or the gut load diet can affect the weight gain of insectivores, as was shown for the Wyoming toad (*Bufo baxteri* Porter) (Li et al., 2009). In that specific case, a lower weight gain coincided with decreased consumption, which in turn might be caused by decreased palatability due to the vitamin powder (Li et al., 2009). A study of Puerto Rican crested toads (*Peltophryne lemur* (Cope)), compared the direct oral application of vitamin A with gut loading and dusting in house crickets. Retinol blood values were higher in toads offered dusted crickets (McComb, 2010). Why the other methods were less effective is unclear. Possibly, retinol is better absorbed when ingested with food, as dietary fat can enhance the absorption of fat-soluble nutrients such as retinol. Hence, the fat in the crickets may have increased retinol absorption compared to direct oral supplementation.

18.7.3 Feeding nutrient enhanced diets during growth

It is now well established that select nutrient concentrations in insects can be altered by manipulating the content of the diet. This is different from gut-loading in that the nutrient changes are not a result of the residual food in the gastrointestinal tract, but rather the nutrients are incorporated into the tissue of the insect. While not effective for all nutrients and for all insect species, nutrients that can be significantly altered using this technique in some species include fatty acids, calcium, carotenoids, and vitamin E.

18.7.3.1 Fatty acids

Early research indicated that the fatty acid composition of insects partially reflects their diet (Cookman et al., 1984; Madariaga et al., 1971; Schaefer, 1968). More recently the fatty acid composition of a number of insects, commonly fed to captive insectivores, has been modified to increase either the levels of omega-6 or omega-3 fatty acids. This includes enhancing the omega-3 content of yellow mealworms (Dreassi et al., 2017; Fasel et al., 2017; Finke, 2015a, Oonincx et al., 2019; van Broekhoven et al., 2015), lesser mealworms (Oonincx et al., 2019), house crickets (Finke, 2015a, Komprda et al., 2013; Oonincx et al., 2019), Jamaican field cricket (*Gryllus assimilis*) (Starčević et al., 2017), superworms (Finke, 2015a), waxworms (Finke, 2015a), silkworms (Chieco et al., 2019) and black soldier fly larvae (Ewald et al., 2020; Meneguz et al., 2018; Oonincx et al., 2019; Spranghers et al., 2017; St-Hilaire et al., 2007). The amount of omega-3 fatty acids and the omega-6 to omega-3 ratio have been implicated as being beneficial in many species due to their role in cell membrane function, gene expression, and inflammation (Schmitz and Ecker, 2008). It is unclear if they might confer similar benefits to insectivores.

18.7.3.2 Calcium

Most insects have a low calcium content. However, as mentioned previously, larvae of both the face fly and the black soldier fly can contain significant amounts of calcium. For black soldier fly larvae the calcium content depends on the diet, with calcium levels ranging from 0.84% to 8.29% (dry matter), with calcium to phosphorus ratios of 1.1:1 to 4.7:1 (Liland et al., 2017; Schmidt et al., 2019; Tschirner and Simon, 2015). These data include both calcium in the insect body exoskeleton and dietary calcium residing in the gastrointestinal tract. Calcium levels in black soldier fly prepupae, which have emptied their gut prior to pupation ranged from 0.12% to 6.61% (dry matter) with calcium to phosphorus ratios of 0.3:1 to 14.9:1 (Spranghers et al., 2017; Wang et al., 2020).

18.7.3.3 Carotenoids

Insect diets rich in carotenoids increase the carotenoid levels in the insect's tissues. However, the amounts retained depend on the carotenoid in question, the dietary levels, and the insect species. Feeding a diet rich in β-carotene increases β-carotene levels in house crickets, migratory locusts, mealworm larvae and superworm larvae (Finke, 2015a; Oonincx and van der Poel, 2011). In contrast, a diet containing β-carotene did not result in any β-carotene retention in blow flies (*Calliphora*) (Vogt and Kirschfeld, 1984), fruit flies (Giovannucci and Stephenson, 1999), and waxworm larvae (Finke, 2015b) and only low levels in silkworm larvae (Chieco et al., 2019). This difference is likely a result of the chromophore the insect uses for vision. While some insects like dragonflies use both retinal and 3-hydroxy retinal as their chromophore most other insects use either retinal or 3-hydroxy retinal (Smith and Goldsmith, 1990). Orthoptera (including crickets and locusts), Coleoptera (including both mealworms and superworms) and Blattodea (roaches) use retinal which is synthesized by cleaving one molecule of β-carotene into two molecules of retinal. In contrast Lepidoptera (including waxworms, butterworms, silkworms and hornworms) and Diptera (including fruit flies and black solider flies) use 3-hydroxy retinal as their chromophore which is synthesized from zeaxanthin. For insects that use 3-hydroxy retinal as their chromophore, dietary β-carotene is first converted to zeaxanthin and then to 3-hydroxy retinal (Giovannucci and Stephenson, 1999; Voolstra et al., 2010). As such it is important to know if the insect being used as food uses retinal or 3-hydroxyretinal as their chromophore since β-carotene has vitamin A activity while zeaxanthin does not.

18.7.3.4 Vitamin E

Increased dietary vitamin E increases the vitamin E content of insects. This was shown for both black soldier fly larvae and mealworms. However, as those larvae were not fasted prior to analysis, at least part of this increase was due to dietary vitamin E remaining in their gastrointestinal tract (Liland et al., 2017; Pennino et al., 1991). Data for house crickets, mealworms, superworms and waxworms, which were fasted prior to analysis, indicates that vitamin E levels also increase in their tissues when fed diets containing high levels of vitamin E (Finke, 2015a). This is similar to swine, where high levels of dietary vitamin E result in enhanced levels in various tissues (Asghar et al., 1991).

18.8 Other considerations

18.8.1 Pathogens/parasites

There is little information regarding commercial feeder insects as a source of pathogens. House crickets from five commercial suppliers in the United States were shown to be free of Oxyurids/pinworms but little other information is available regarding insects as a source of parasites for insectivores (Klarsfeld and Mitchell, 2005). One exception is a study in which black soldier fly larvae were provided with either coccidian parasites [*Eimeria tenella* (Tyzzer) or *Eimeria nieschulzi* (Dieben)] or nematode [*Ascaris suum* (Goeze)] eggs (Muller et al., 2019). After 10 days all three parasites were recovered from the outside of the black soldier fly larvae and prepupae. Moreover, these parasites were also recovered from the intestines of the larvae and, in part, from the intestines of the prepupae indicating that they could vector these parasites.

Several studies have been done on the vectoring capacity of insects regarding pathogenic bacteria. The lesser mealworm can vector *Salmonella spp*. with both larvae and adults carrying *Salmonella spp*. both externally and internally (Crippen et al., 2009). Additionally, *Salmonella* was detected in newly emerged adults from infected larvae suggesting some bacteria are carried through metamorphosis (Crippen et al., 2012). Moreover, while black soldier fly larvae fed chicken manure inoculated with *Salmonella enterica* (Kauffmann and Edwards) Le Minor & Popoff (6.9 log CFU/g) reduced the concentration of *S. enterica* in the manure, populations were still present at 3.3 log CFU/g after three, and at 2.2 log CFU/g after six days (Erickson et al., 2004). Thus, it seems likely that these, and other insect species, could vector *Salmonella spp*. and other pathogenic microorganisms. Therefore, these species and others should be obtained from qualified suppliers, fed appropriately, and maintained hygienically, to minimize potential transmission.

18.8.2 Heavy metals

Insect species differ in how they cope with heavy metals. Whereas differences between species exist, cadmium accumulation is consistently suggested for several species of flies, including houseflies (Charlton et al., 2015), fruit flies (Maroni and Watson, 1985), flesh flies (*Sarcophaga peregrina* Robineau-Desvoidy) (Gao et al., 2017) and black soldier fly larvae (Diener et al., 2015; Gao et al., 2017; Purschke et al., 2017; van der Fels-Klerx et al., 2016; Wu et al., 2020). In contrast, mercury, chromium, and arsenic are largely excreted by black soldier fly larvae (Biancarosa et al., 2018; Diener et al., 2015; Gao et al., 2017; van der Fels-Klerx et al., 2016). It seems that lead and zinc accumulate in the outer layer of the larval exoskeleton when the larvae are exposed to higher dietary concentrations (Diener et al., 2015). For another group of commonly used feed insects, the Orthoptera, cadmium accumulation also seems to be a risk factor. This was shown for seven different acridid species and in the Jamaican field cricket (Bednarska et al., 2015; Devkota and Schmidt, 2000a, 2000b; Zhang et al., 2012). However, a study with house crickets indicates that cadmium from a pulse dose can be rapidly excreted (Van Hook and Yates, 1975). In the yellow mealworm dietary cadmium does not seem to accumulate, however dietary arsenic does (van der Fels-Klerx et al., 2016; Vijver et al., 2003). Essential elements such as copper, zinc and iron seem carefully regulated in the yellow mealworm (Keil et al., 2020; Vijver et al., 2003). This seems to be a general trend for essential elements in insects, for instance, zinc is carefully regulated in the Jamaican field cricket (Bednarska et al., 2015), as is zinc in larvae of the black soldier fly (Diener et al., 2015). The latter species loses excess zinc via ecdysis. A similar method seems to be used by yellow mealworms which stores cadmium in their exoskeleton and can thereby decrease cadmium concentrations via molting and metamorphosis (Lindqvist and Block, 1995; Lindqvist and Block, 1997). Increasing levels of dietary zinc aid in reducing cadmium accumulation (Keil et al., 2020). Such mechanisms help the insect cope with exposure to heavy metals. Still, the presence of heavy metals in the insect's diet should be avoided as these can transfer to the consuming insectivore, be it via storage in the insect body, or via the food remaining in its gastrointestinal tract.

18.8.3 Mycotoxins

Ingredients commonly used in commercial insect diets can become contaminated with various species of molds which can produce mycotoxins. Several studies have been done to determine the effects on commonly reared insect species. For the yellow mealworm, the following mycotoxins were investigated: aflatoxin B1 (Bosch et al., 2017) deoxynivalenol (Ochoa Sanabria et al., 2019; van Broekhoven et al., 2017), and zealerone (Niermans et al., 2019). A similar list is available for the lesser mealworm: aflatoxin B1 (Camenzuli et al., 2018), deoxynivalenol (Camenzuli et al., 2018 Leni et al., 2019), fumonisin 1 and 2 (Leni et al., 2019), ochratoxin A (Camenzuli et al., 2018), and zearalenone (Camenzuli et al., 2018; Leni et al., 2019). The black soldier fly has also been extensively tested with aflatoxin B1 (Bosch et al., 2017; Camenzuli et al., 2018; Purschke et al., 2017), aflatoxin B2 (Purschke et al., 2017) deoxynivalenol (Leni et al., 2019; Camenzuli et al., 2018; Purschke et al., 2017), fumonisin 1 and 2 (Leni et al., 2019), ochratoxin A (Camenzuli et al., 2018; Purschke et al., 2017), and zearalenone (Camenzuli et al., 2018; Leni et al., 2019 Purschke et al., 2017). Unfortunately, quantitative studies on Orthoptera are currently not available, although these are to be expected in the coming years.

The results obtained in the aforementioned studies are remarkably consistent: (1) mycotoxin exposed insects developed and grew normally; (2) mycotoxins do not accumulate in the insect body; (3) low concentrations are sometimes detected, probably due to contaminated feed present in the gut; (4) mycotoxins are catabolized by the insect, but generally not to the better known and often also toxic metabolites. As there is the possibility that metabolites that have not been identified and detected in these insects are toxic, further insights into the exact pathways are needed. For now, however, there seems to be little risk of mycotoxins for insectivores.

18.8.4 Other toxins

It is well known that many species of insects sequester toxic compounds from their diet making them unpalatable or even toxic to certain insectivores. In the wild, these species are generally brightly colored (aposomatic) to warn potential predators of the consequences of feeding on these species. Monarch butterflies (*Danaus plexippus* L.) and milkweed bugs (*Oncopeltus fasciatus* Dallas) are but a few of the many species that sequester toxins from their feed (Berebaum and Miliczky, 1984; Brower, 1969). Since most captive-raised feeder insects are fed diets containing commercial feed ingredients also used for domestic animals, it seems unlikely that they would accumulate few if any toxins from their diet as long as the diet was properly made and stored.

Almost nothing is known regarding the potential antinutritional properties of insects except for thiaminase. Thiaminase is an enzyme that when ingested splits thiamine (vitamin B_1) effectively destroying its vitamin properties. While thiaminases are typically associated with certain species of fish (Spitzer et al., 1941) it has been reported in both domesticated silkworm larvae (*B. mori*) and African silkworm pupae (*Anaphe* spp), although the levels found in domesticated silkworm larvae were only one-third of those found in African silkworm pupae (Nishimune et al., 2000; Watanabe et al., 2001). These authors noted that in addition to thiamine, pyridoxine, taurine, and possibly other nutrients could also serve as a substrate for this enzyme. The consumption of *Anaphe* pupae has been associated with seasonal ataxia in humans in Nigeria, presumably due to thiamine deficiency (Adamolekun, 1993; Adamolekun et al., 1997). The extent to which thiaminases are found in other species of insects, and their potential effect on insectivores, is currently unknown.

18.8.5 Uric acid

Uric acid is produced by most insect species as a nitrogenous waste product from protein and purine metabolism. Some species, most notably cockroaches, store significant amounts of this nitrogen source in their fat body where bacterial endosymbionts can use it for amino acid synthesis (Patino-Navarrete et al., 2014). The amount of uric acid stored by cockroaches depends on the species, age, sex, and most importantly diet (Mullins and Cochran, 1975a, 1975b). Uric acid levels in 23 cockroach species, determined with a uricase-spectrophotometric method, were up to 30% of the roach's dry weight, with an average value of 12.8% (Mullins and Cochran, 1976). Twenty of these species were fed commercial dog food containing 25% protein, which could partially explain these high levels. In another study, using the same method, house crickets contained only 2.6% uric acid on a dry matter basis (Cochran, 1976). More recently, uric acid concentrations measured by reverse-phase HPLC were between 4.8% and 7.0% dry matter for discoid cockroaches (*Blaberus discoidalis* Serville), and between 1.0% and 1.2% dry matter for house crickets (Sabolova et al., 2021). Whereas the reported values were lower, the contrast between these species was similar. Desert locusts contained only 0.44%−0.75% of their weight (dry matter) as uric acid. In some animal species a build-up of uric acid in the body, can lead to gout (Mader, 2006). Hence, insectivores that may be prone to develop gout should primarily be offered insects with low uric acid contents.

18.9 Conclusions

Insects are a good source of many nutrients although for most nutrients the values vary widely depending on the insect species, the life stage, the diet and the conditions in which they are raised. In general, most species are good sources of amino acids, fatty acids, most minerals and most B vitamins. Based on analysis of feeder insects and reports of nutrient deficiencies in captive insectivores the nutrients of concern in a captive insectivore feeding program are calcium, and fat-soluble vitamins A, D and E. As such, captive insectivores should be fed a mix of invertebrates that have been dusted, gut-loaded or fed nutrient enhanced diets during growth to provide a wide range of nutrient intakes to reduce the risk of nutrient deficiencies.

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