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RESEARCH ARTICLE

Bugbook: nutritional requirements for edible insect rearing

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Abstract

The rapid expansion of insect farming as a sustainable approach for protein production has led to an accumulation of published research on the use of low-value substrates as insect feed. However, compared to conventional livestock, there is a lack of fundamental knowledge about the nutritional physiology and requirements of insects. Such knowledge can lead to the development of optimised feeding substrates that boost insect performance and nutrient composition. In this chapter, the current knowledge on the nutritional requirements of the most relevant insects produced for food and feed is reviewed, and the methods and assays used to investigate the specific requirements are presented. This description of current knowledge on nutritional requirements of edible insects can be used to design suitable rearing diets and as a foundation to further our understanding of insect nutrition.

Keywords

Coleoptera – Diptera – edible insects – nutrient requirements – Orthopera

1 Introduction: background and motivation

Edible insects can play a pivotal role in recycling organic by-products and biowastes, and their products provide useful raw materials for various applications in the bioeconomy (Kee *et al.*, 2023). Examples include the production of whole insects, insect protein, fat, and specific functional extracts for use in food (de Carvalho *et al.*, 2020), feed and pet food (Gasco *et al.*, 2020), the use of lipids as biofuels (Li *et al.*, 2011), chitin

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and its derivatives in packaging materials, and frass as plant fertiliser (Beesigamukama *et al.*, 2022; Le *et al.*, 2023). While up to 2,000 species of insects are described as edible (Kim *et al.*, 2019), production of edible insect species focusses on a limited number of species. Currently in focus are: the black soldier fly (*Hermetia illucens* (L.); Diptera: Stratiomyidae), the housefly (*Musca domestica* (L.); Diptera: Muscidae), the silkworm (*Bombyx mori* (L.); Lepidoptera: Bombycidae), the yellow mealworm (*Tenebrio molitor* (L.); Coleoptera: Tenebrionidae), the house cricket (*Acheta domesticus* (L.); Orthoptera: Gryllidae), and the migratory locust (*Locusta migratoria* (L.); Orthoptera: Acrididae), with the last three being approved as "Novel Foods" in the EU (EFSA *et al.*, 2021a,b, 2022). They can reduce the environmental impact of human diets if consumed directly as an alternative to conventional livestock products (van Huis and Oonincx, 2017), and their ability to acquire their nutrients from low-value substrates is fundamental to their sustainability (Smetana *et al.*, 2019).

Feedstock supply is an important driver of environmental impact and currently makes up 50-70% of the operational costs in conventional livestock farming (Spring, 2013). Production efficiency in these systems has increased considerably over the last decades, due to improved, genetics, nutrition, and disease control (Thornton, 2010). This progress was partially due to the characterisation of feed ingredients via standardised protocols, combined with improved understanding of animal physiology, digestive systems, the microbiome, and metabolism. This included determining feed digestibility metrics per species, and nutrient requirements per breed and development phase. By matching the knowledge on feed characteristics with nutritional requirements of target animals, the performance of livestock can now be predicted with high accuracy. Similar to conventional livestock farming the feed used in insect farms has a tremendous impact on the economic performance, and on the zootechnical and environmental efficiency of the production (Oonincx, 2021). Conversely, feedstock formulation for edible insects is in its infancy. While requirements for macronutrients (proteins, lipids, carbohydrates) received some attention (Bellezza Oddon *et al.*, 2022, 2024), little information is available on vitamins and micronutrients. Moreover, the lack of standardisation of methodologies for assessing nutrient utilisation in edible insects is a key challenge (Bosch *et al.*, 2020; Deruytter *et al.*, 2025). This is complicated by the diversity of feedstocks, feeding environments, digestive physiology and capacities, and plasticity among edible insect species often intertwined with microbial processes (Auger *et al.*, 2025). Feeding insects with high proportions of nutrient-rich human-edible ingredients would support high productivity, as was the case for conventional livestock. However, this practice should not be replicated in insect production as it is unsustainable. Instead, the focus should be on maximising nutrient utilisation of by-products and available biowastes.

The goal of this article is to summarise best practices and methods used to determine nutritional requirements, and the nutritional value of ingredients and diets for edible insects. Such methods are required to generate data that can form the foundation of feed evaluation systems as well as formulating diets for edible insect production. It focuses on the larval and nymphal life stages, with some links to adult insects. However, the field of edible insect nutrition is rapidly developing. The methods described below provide a current summary and directions for further method development.

2 Basics of nutritional requirements of animals

Animals require nutrients and energy for various metabolic processes in the body throughout the different phases of life. These are used to maintain body functioning ('maintenance processes'), whereas additional nutrients and energy are needed for 'production processes' such as the larval growth and egg production in adults (Cheeke and Dierenfeld, 2010). In general, insects require the same ten amino acids considered essential for mammals (arginine, lysine, leucine, isoleucine, tryptophan, histidine, phenylalanine, methionine, valine, and threonine). Moreover, sterols, various minerals, and eight B-complex vitamins (thiamine, riboflavin, nicotinamide, pyridoxine, pantothenate, folic acid, biotin, and choline) are essential parts of insect diets (Cohen, 2003). Furthermore, vitamin C is required by many insects consuming fresh plant matter, and for some species also carotenoids are needed. Animal species obviously differ in their dietary requirements and tolerances as their digestive and metabolic capacities have adapted to their ecological niche throughout evolution. Within species, requirements can further depend on genetic variation, life stage, body weight, and sex. Moreover, as the environment (e.g. temperature, sanitation, space) can have a profound impact on animal functioning, it follows that the quantitative requirements of animals are actually not fixed but are conditional for the specific situation in which the requirements were quantified for a given population.

The nutrients and energy that farmed animals require to optimally perform with minimal impact on the environment should be supplied via the diet or feed. Feed evaluation methods have been developed to match nutrient supply with requirements. Such methods aim to quantify the bioavailable energy and nutrients in feeds and feed ingredients. Bioavailability is defined as the amount of a nutrient released from ingested feed, hydrolysed, absorbed by the gut epithelium and distributed to body tissues in a chemical form that can be used for the metabolism of the animal (Grundy *et al.*, 2024). The content of bioavailable energy and nutrients of raw materials largely determine their feeding value, but it is tedious and costly to assess. Bioavailability is generally approximated via digestibility, which is defined as the amount of ingested energy or nutrients that disappears in the gastrointestinal tract (Grundy *et al.*, 2024). Other aspects of feeding value are potential anti-nutritional factors that might negatively impact digestive processes and, therefore, bioavailability. Data on the nutritional value of raw materials can ultimately be transferred to feeding tables combined with costs, and used to formulate complete diets for supporting optimal performance of the target animals in their specific environment at minimum costs. Apart from being complete and balanced, diets can be formulated to optimise raw material use, while minimising costs and environmental impact (Van Lingen *et al.*, 2018). For example, although amino acids can be metabolised to yield energy, this is generally undesirable as proteins are a more expensive source of energy than carbohydrates and lipids, and their metabolism yields in nitrogen-containing metabolites that when excreted pollute the environment (Castillo *et al.*, 2000). Hence, nutritionists typically aim to balance amino acids and energy in formulated diets. Hence, the importance of having methods to quantify animal requirements and to evaluate the nutritional value of raw materials is apparent. The nutritional value depends on chemical composition (i.e. nutrients and anti-nutritional factors), and physical properties like particle size, water-holding capacity, substrate texture, and water content.

Nutrient requirements of animals in various developmental stages have been studied in particular between the 1940s and 1970s. Typically, synthetic diets containing relatively pure chemical components were used to allow controlled changes in the provision of the individual nutrient of interest and determine its threshold values. Outcomes of these studies are collated in several nutrition reference books of the National Research Council (e.g., NRC, 1994, 2012). Various national institutes and companies have complemented the knowledge with that of data obtained under more practical conditions leading to more detailed estimates for use in practice. Feed evaluation systems are available for livestock animals at various life-stages with those for pigs being highly developed. For pigs, nutrient and energy requirements are known both for maintenance and production processes including requirements for the specific stages of growth in young animals, as well as how living conditions like sanitary status might affect requirements. Tolerances for components potentially present in raw materials that might impair performance are known and controlled for by setting maximum inclusion levels. Feeding tables include nutrient bioavailability estimate values for the vast majority of available raw materials. Methods to approximate nutrient bioavailability such as the ileal digestibility assay for amino acids have been developed (Stein *et al.*, 2007) and are widely used e.g. INRAE-CIRAD-AFZ (2024). Equations have been developed to approximate the energy value of feedstuffs based on their organic components including digestible and fermentable carbohydrates, digestible proteins and digestible lipids.

The steps described above and approaches used in research on other animal species are instrumental to design studies to advance insect nutrition. Moreover, considering that insects often live directly in the substrates they consume and these substrates harbour active microbiota that change the chemical composition, approaches from other fields such as industrial biotechnology (e.g. bioprocess engineering) are relevant as well.

3 Current knowledge on nutritional requirements of edible insects

Insects exhibit enormous genetic diversity, and the genetic distance between different insect orders is considered greater than between vertebrate species (Trautwein *et al.*, 2012). This diversity reflects on their nutritional physiology and requirements. This review, therefore, focuses on species of three orders relevant for applications in food or feed.

Diptera: Black soldier fly and housefly

The housefly and the black soldier fly (BSF) are currently the most important Diptera for application as food, feed, waste treatment, and production of other high-value applications (Kaczor *et al.*, 2023; Sanchez *et al.*, 2022; van Huis *et al.*, 2020; Van Zanten *et al.*, 2015). Past studies have suggested that research on the well-studied common fruit fly (*Drosophila melanogaster*, Diptera: Drosophilidae) and other less-studied flies such as the green bottle fly larvae (*Lucillia sericata*, Diptera: Calliphoridae) and stable fly larvae (*Stomoxys calcitrans*, Diptera: Muscidae) can provide insights into nutrient requirements of the most important Diptera in insects as food and feed (Gold *et al.*, 2018). At the same time, while general trends on nutritional requirements may apply, differences in ecological niches and evolutionary history suggests differences between dipteran species. Research on the nutritional requirements first started in the 1950s for housefly larvae by evaluating amino acid and vitamin needs (Chang and Wang, 1958; House and Barlow, 1958). As interest in the use of BSF grew in the last two decades, so did the number of studies in their dietary requirements. Studies on their nutritional requirements first focused on macro-nutrients (Barragan-Fonseca *et al.*, 2021; Oonincx *et al.*, 2015) followed by more in depth studies on amino acid requirements (Lemme and Klüber, 2024). In both species, studying nutritional requirements is challenging compared to conventional livestock as they live within their moist feed and interact with the present microbiota (Wynants *et al.*, 2019). For an in depth discussion on microbial interactions see (Auger *et al.*, 2025). While these interaction with bacteria, viruses, fungi, and archaea are recognised, their specific contribution to larval nutrition is yet to be quantified under various biotic and abiotic conditions. These microbes can be a direct nutrient source for Dipteran larvae, pre-digest nutrients and produce essential nutrients, or compete for nutrients with larvae (Pisa *et al.*, 2024; Storelli *et al.*, 2011, 2018). Together the larval and microbial metabolism continuously changes substrate characteristics such as pH (Klammsteiner et al. 2020, Gold et al., 2020), moisture content, chemical composition, temperature, and both the larvae, and the microbial community themselves (Wynants et al., 2019).

Dipteran larvae are currently farmed for use as feed ingredients. In order for this practice to be sustainable and economically viable, their substrate would need to consist of by-products with a low value, and with no or limited direct application as livestock feed ingredients. About 16-20% crude protein on a dry matter (DM) basis facilitates the most efficient and fastest growth in BSF (Barragan-Fonseca *et al.*, 2021; Bellezza Oddon *et al.*, 2022). Lower dietary protein concentrations allow BSFL growth, albeit slower (Ewusie *et al.*, 2018). Higher protein concentrations, such as those found in meat and slaughterhouse waste, limit growth and development (Gobbi *et al.*, 2013; Gold *et al.*, 2020). While these animal-derived ingredients are unsuitable as complete diets, animal-derived protein is often more suitable than plant-derived protein due to a more favourable amino acid profile (Woods *et al.*, 2019). Indeed, understanding protein requirements, requires qualitative and quantitative insight in essential amino acid requirements. While for the housefly the aforementioned ten amino acids were shown to be essential, further quantitative understanding is still needed as larval growth was retarded when only those were provided (Chang and Wang, 1958). For BSF larvae the first steps towards determining amino acid requirements have been taken. For instance, Sandrock (2024) identified lysine as the first limiting amino acid and arginine, isoleucine, tryptophan and threonine as co-limiting in neonatal diets based on chicken feed. While protein content did not influence their survival, the study showed the relevance of physical properties beyond equal particle size (≤1 mm), such as structural components and water-holding capacity. Similar results on amino acid requirements were reported for fattening BSF larvae, including a rather indifferent role of methionine (Berggreen, 2024). Yet somewhat conflicting results in growing BSF larvae, particularly for lysine, might indicate differences between developmental stages (Koethe *et al.*, 2022). Indeed requirements of neonate larvae (1st/2nd instar) might differ from growing and fattening larvae as reflected in changes in body composition during larval development (Liu *et al.*, 2017). Additionally, factors such as the conspecific genetic diversity can also influence quantitative requirements (Sandrock *et al.*, 2022)

Carbohydrates are typically the largest constituent of larval diets (Gold *et al.*, 2018). These can be divided into digestible and non-digestible carbohydrates (fibres). Currently utilised biowastes are particularly high in fibres (Peguero *et al.*, 2021). These are indigestible, as larvae lack the enzymes to break the linkage bonds. Hence, large amounts of fibres reduce nutrient density, which can reduce larval growth. Conversely, digestible carbohydrates (for instance starch) contain bonds which can be digested by enzymes such as amylases, present in the digestive tract of Dipteran larvae (Bonelli *et al.*, 2019; Pimentel *et al.*, 2018). These then provide energy, which can be stored as lipids. Hence, larvae produced on diets low in protein and high in carbohydrates will have a higher lipid content than those produced on diets more balanced in carbohydrates and proteins (Barragan-Fonseca *et al.*, 2018). Besides chemical composition, also larval performance is influenced by the ratios between of proteins and carbohydrates

(Barragan-Fonseca *et al.*, 2019; Cammack and Tomberlin, 2017; Eggink *et al.*, 2023).

Specific requirements for lipids are understudied in houseflies and BSF. In many natural diets (e.g. fruit and vegetables, manures) lipids are a minor constituent. Larvae can acquire energy and synthesise lipids from digestible carbohydrates and proteins. Hence, they can thrive on low-lipid substrates, if sufficient digestible carbohydrates are available (Oonincx *et al.*, 2015). Inclusion of varying levels of alpha-linoleic acid (2.5–30% of total fatty acids) in diets influences body composition of BSF, but does not impact survival or rate of development (Oonincx *et al.*, 2020). Whether larvae require a minimum dietary concentration of this omega 3, or for omega 6 fatty acids, requires further investigation. Also, requirements for other micronutrients, such as minerals and vitamins are understudied. Historical literature indicates that b-vitamins such as thiamine, riboflavin, pantothenic acid, nicotinic acid, choline, pyridoxine, and biotin are required by housefly larvae (House and Barlow, 1958). While most of these are needed during early larval development, choline and pyridoxine are required for successful pupation (House and Barlow, 1958).

Coleoptera: Yellow mealworm and the lesser mealworm

The yellow and lesser mealworm (*Alphitobius diaperinus* (Panzer); Coleoptera: Tenebrionidae) are the commercially most interesting species within the Tenebrionidae. Both species are pests in grain storage and processing facilities, while the lesser mealworm is also considered a pest in poultry stables (Veldkamp *et al.*, 2021).

Nutritional requirements of yellow mealworm larvae were first reported in 1950 and highlighted their preference for carbohydrate rich diets (80–85% DM) (Fraenkel, 1950). Two decades later this was followed by more detailed studies on protein and carbohydrate requirements (Davis, 1969, 1970, 1971a). More recently, further studies addressing protein and carbohydrate requirements of yellow mealworm larvae were published. Some used by-products as ingredients (Mancini *et al.*, 2019; Oonincx *et al.*, 2015; Rumbos *et al.*, 2020, 2021; Urrejola *et al.*, 2011; Van Broekhoven *et al.*, 2015), while others used synthetic diets (Morales-Ramos *et al.*, 2010, 2011, 2013; Rho and Lee, 2014). Wheat bran is commonly used as a diet for yellow mealworm. Gradually replacing this by pea or rice protein, creating diets ranging in protein content (10-80% DM), showed reduced growth when the substrate had >20% protein (Kröncke and Benning, 2023). Similarly, yellow mealworm larvae fed legume flours with protein contents ranging from 23 to 42% (DM) grew poorly, while durum wheat flour or white flour with protein contents of 11–14% (DM), and high in digestible carbohydrates more efficiently supported larval development (Rumbos *et al.*, 2020). In a study comparing two sets of compound diets (either 17 or 20% protein (DM)) apparent differences in larval survival and development were unexplained by the difference in dietary protein content (Rumbos *et al.*, 2022). In the aforementioned study replacing wheat bran with rice or pea protein, rice protein resulted in better performance than pea protein (Kröncke and Benning, 2023). Clearly, high dietary protein does not necessarily guarantee good larval performance and other factors such as protein digestibility, and presence of antinutritional factors can affect larval growth and development.

Besides protein content, the ratio between protein and metabolisable energy is an important factor in animal performance. Digestible carbohydrates are a primary energy source for yellow mealworm larvae. The optimal protein-to-carbohydrate ratio lies between 1:1 and 1:3, whereas larval growth is reduced in heavily protein- or carbohydrate-biased diets (Morales-Ramos *et al.*, 2020b; Rho and Lee, 2022). The nutritional requirements of adults and dietary effects on survival and reproductive output is less studied. A slightly carbohydrate-biased ratio seems preferred by adults (Rho and Lee, 2014, 2016).

Similar to housefly larvae, the same 10 amino acids essential for growth in rats and other vertebrates are essential for yellow mealworm larvae (Davis, 1969, 1971b, 1974; John *et al.*, 1978, 1979). The amino acids showed that serine, tyrosine, glutamic acid and possibly glycine are non-essential for growth, while alanine, cystine, proline and aspartic acid are partly indispensable (Davis, 1975). The optimal dietary concentration of essential amino acids reflects the amino acid profile of the larvae, except for phenylalanine which sufficed at a lower relative concentration (50%), and threonine and tryptophan which seem to be required at a higher concentration (>200%) (John *et al.*, 1979). Contrary to yellow mealworms, studies on nutrient requirements of lesser mealworm larvae are scarce. In general, a dietary protein content above 20% favours larval growth (Kotsou *et al.*, 2021; Ricciardi and Baviera, 2016). Adding dry instant yeast to wheat bran, and thereby elevating protein contents from 17 to 30%, increased larval growth, weight, and feed efficiency (Kotsou *et al.*, 2021).

Orthoptera: The migratory and desert locusts, and the house cricket

The nutrition of herbivorous orthopterans such as crickets and locusts has received considerable attention in the past. For locusts, most early studies were conducted in the light of targeted pest management strategies and the prediction of outbreaks (Cease, 2013). These studies included aspects such as nutritional requirements and feeding behaviour. Based on this information, colonies could be setup and management strategies could be tested. Similarly, the basis of cricket nutritional studies was laid in the sixties and seventies of the last century. There are a few general considerations relevant for studying Orthopteran nutrition. For instance, locusts and crickets cannibalise on each other if, in addition to other factors, diets are insufficient or unsuitable which could potentially mask nutrient deficiencies in individuals (Dadd, 1961a). Moreover, given that water is often provided separately when conducting trials, it is relevant to use proper materials. Water can be provided via a water pad filled with cotton wool, or a metal sponge (Dadd, 1960a). The cotton wool might be partially consumed providing nutrients while the metal sponge might leach minerals into the water, hence the method of water provision should be made based on the nutrient(s) under study. While crickets and locusts are both Orthoptera, there are large apparent differences. For instance, their moment of activity, social behaviour, and feed preferences. Hence, locust and cricket nutrition are discussed separately.

In the early sixties a basic artificial synthetic diet was developed for migratory locusts and desert locusts (*Schistocerca gregaria* (Forskål); Orthoptera: Acrididae) (Dadd, 1960a). This diet consisted of cellulose, sucrose, dextrin, a salt mixture, cholesterol, linoleic acid, casein, peptone, egg albumen, ascorbic acid, and ten water soluble vitamins of the B complex. It was then used to test various aspects of locusts nutrition. For example, establishing minimal requirements of vitamins for desert locusts. While both species are foliage feeders, they do not require chlorophyl (Dadd, 1961b). Given that locusts do not live in their substrate, real-time observation of their diet preference and feeding behaviour are more possible. The amount of feed consumed can vary based on its nutrient concentration. For instance, fifth instar nymphs of the migratory locust provided with a diet based on casein, sucrose and cellulose increased their feed intake by 44% on a diet with 14% protein, compared to one with 28% protein content (Simpson and Abisgold, 1985). This increased feed intake was primarily due to a shorter time between feedings and not by increasing the amount eaten per feeding. In the same study, digestible carbohydrates were replaced by cellulose, but this did not affect feed intake. While in theory several ingredients can serve as a protein source, not all have proven to suffice. For instance, casein as a sole source of protein did not allow proper growth in desert locusts and addition of single amino acids, or nucleic acids, did not solve this issue (Dadd, 1961a). However, when egg albumin with peptone were used, proper growth was attained.

Available carbohydrates are another important element of locust diets. In their absence, locusts fail to develop even when an energy source in the form of fatty acids and glycerol is available (Dadd, 1960c). While 13% of sucrose or glucose support growth during the early instars, for further development at least 26% of digestible carbohydrates is needed for adequate growth and development. This suggests that carbohydrate requirements differ quantitatively over nymphal stages. Clearly, the form of carbohydrates affects their suitability; most hexoses, dextrin, oligosaccharides, and starches, suffice, while some pentoses do not. Moreover, there seem to be differences between migratory and desert locusts; for instance galactose and lactose work poorly in migratory locusts but better in desert locusts. A balanced (1:1) carbon-to-protein ratio has been described to be optimal as described for the migratory locust (Chambers *et al.*, 1995). In addition to digestible carbohydrates, fibres (e.g. cellulose) seem to be an important additive to artificial diets for their role as a bulking agent (Dadd, 1960a)

The lipid requirements for developing to adulthood of both locust species can be fulfilled with a combination of cholesterol and either linoleic acid (C18:2 n6) or linolenic acid (C18:3 n3) (Dadd, 1960a; b). Arachidonic acid (C20:4 n6) can be utilised to support growth, but leads to wing deformations. While qualitatively similar, migratory locusts are more prone to deficiency symptoms than desert locusts.

Vitamin A, as such, is not required by locusts. Provitamin A carotenoids, however, do play a relevant role. Beta-carotene, for instance, is required for pigmentation in desert locusts and improves growth and development (Dadd, 1957, 1960a). The provision of carrots increases the contents of α- and β-carotene in nymphs of the migratory locust, whereas this effect seems absent or negligible in adults (Oonincx and Van der Poel, 2011). Locusts also require various B-vitamins; that is, thiamine, riboflavin, nicotinic acid, pyridoxine, folic acid, meso-inositol, calcium pantothenate, biotin, and choline chloride are dietarily required to facilitate growth and development (Dadd, 1960a). Moreover, vitamin C (ascorbic acid) is an essential nutrient for desert locusts (Dadd, 1957, 1960a). In its absence these locusts often die during moulting. No dietary requirement for vitamin D is established for locusts. At least migratory locusts can synthesise it *de novo* when exposed to UVb radiation (Oonincx *et al.*, 2018). Vitamin E (Tocopherol) does not seem to be required by desert or migratory locusts (Dadd, 1960a).

Also house cricket nutrition was historically studied. They can develop on artificial diets consisting of casein, glucose, cholesterol, a mixture of inorganic salts, and a combination of B-vitamins (McFarlane, 1964). Dietary protein levels between 20 and 30%, and carbohydrate levels between 32 and 47% yield the best growth outcomes (Patton, 1967). House crickets can distinguish between sucrose and amino acids, and after a period of amino acid deprivation they will try to increase their amino acid intake (Tierney *et al.*, 2023). High concentrations (>10%) of simple sugars reduce successful development to the adult stage, with effects of fructose being more pronounced than glucose (Francikowski *et al.*, 2021). Trehalose is the dominant sugar in cricket haemolymph and could be preferable in diets (Wang and Patton, 1969). Besides carbohydrates and protein, the house cricket requires dietary lipids for successful reproduction (Meikle and McFarlane, 1965). Early studies indicated that the provision of wheat germ oil, especially rich in linoleic acid, but also linolenic acid, improves early growth compared to pure linoleic acid (Ritchot and McFarlane, 1962). Moreover, egg hatchability increased from 1.1 to 53.6% which could be due to linolenic acid, or another component in the wheat germ oil, such as vitamin E.

As house crickets can convert oleic acid (C18:1n9) to linoleic acid, they might not have a specific requirement for the latter, but can suffice with either of these fatty acids (Crippsc and De Renobales, 1988). The cricket *Teleogryllus commodus* (Walker); Orthopetrea: Gryllidae)can elongate linoleic acid to arachidonic and eicosadienoic acid (C20:2 n6), and can elongate linolenic acid to eicosapentaenoic acid (C20:5 n3) (Jurenka *et al.*, 1988). This might well be true for house crickets as well. While the house cricket can synthesise various fatty acids, including linolenic acid, palmitic acid, and stearic acid (Crippsc and De Renobales, 1988), a dietary source of linolenic acid, could still be advantageous.

The aforementioned artificial diet was used to determine vitamin requirements of house crickets. They require choline and vitamin B_1, B_3, B_5, B_6, and B_7 for optimal growth and survival, with the latter being essential for nymphal development (Ritchot and McFarlane, 1961). Dietary carotenoids are poorly retained, and are seemingly not required by house crickets (Ogilvy *et al.*, 2012) (Veenenbos and Oonincx, 2017). Vitamin E is needed for reproduction (e.g. spermiogenesis and sperm motility (McFarlane, 1974) and fecundity (Prévost and McFarlane, 1980). Increasing levels of vitamin E (4.3-86 mg/kg) can cause dose dependent albinism in adult males on a low copper diet (McFarlane, 1972). Female house crickets require 2 mg of copper/kg, while males require 10 mg/kg (McFarlane, 1974). A recent study that used self-selection of various by-products indicates that vitamin C, manganese and sterols are important for maximising growth, whereas higher levels of vitamins B_1 and, B_5, can limit growth (Morales-Ramos *et al.*, 2020a). More quantitative studies on requirements for amino acids, B-vitamins, and minerals are needed to further understand the nutrient requirements of house crickets.

4 Methods to assess nutrient requirements of edible insects

Nutritional requirements can pertain to different functions and life stages. For instance, during the growth phase parameters of relevance are weight gain and feed efficiency, while in the reproductive phase egg production, egg eclosion rates, and neonate survival are more relevant (Pascual, 2024). Especially in holometabolous insects, adult fitness depends on nutrients stored during immature stages and mobilised in the adult stage (Arrese and Soulages, 2010). Thus, studies on nutrient requirements and their methodological approaches can differ depending on the aim of experiments.

Nutrient requirements by function

Maintenance requirements

Maintenance requires certain nutrients for basal metabolism and activity. These can be determined or estimated by different methods. One method is partial or total fasting trials of the nutrient under study to determine the amount of nutrient used when supply is limited, which helps to estimate maintenance requirements (McDonald *et al.*, 2022). For example, providing a nitrogen-free diet for a short period to determine maintenance protein requirement from the observed protein balance. As the fasting evolves, interactions of amino acid metabolism must be considered (D'mello and Lewis, 1970). Maintenance energy requirements can be determined by balances, or direct and indirect calorimetry (Tartes and Kuusik, 1994). For example, Roe *et al.* (1985) determined these for female house cricket nymphs based on the balance between metabolisable and production energy using an equation proposed by Harris (1966) based on O_2 consumption, CO_2 production, and excretion of N in uric acid. While the most common approach is to determine the overall growth, or reproductive requirements of insects, it may be useful to distinguish between maintenance and production.

Reproduction requirements

Feeding behaviour of hemimetabolous nymphs often resemble those of adults. However, holometabolous insect larvae often exhibit fundamentally different feeding behaviour than adults, as seen in the BSF and yellow mealworms. In these species, larvae are voracious feeders species, while their adults display contrasting feeding behaviours. Historically, adult BSF were considered non-feeding, but it is now well established that they do feed and use extra-oral digestion (Lemke *et al.*, 2023). Currently, adult BSF are rarely fed in farming systems, as this is perceived to not contribute significantly to egg production, whereas the contrary is true for yellow mealworm beetles (Rho and Lee, 2014). This difference is likely due to divergent reproductive strategies; BSF females lay a large egg clutch a few days after adult emergence (Oonincx *et al.*, 2016), sometimes followed by a second smaller clutch, whereas yellow mealworm females continuously lay eggs. Hence, the overall reproductive output correlates with longevity and nutritional status in the latter species. Provision of sugar and/or protein to adult BSF increases longevity, however, reported effects on fecundity vary between no effect, and increased fecundity (Bertinetti *et al.*, 2019; Klüber *et al.*, 2023; Lupi *et al.*, 2019). In adult yellow mealworms, the protein-to-carbohydrate ratio is a significant factor in optimising longevity and total fecundity (Rho and Lee, 2016). While this phase has a minor impact on overall feed consumption, understanding the underlying mechanisms, including the timing of nutrient provision, is essential to optimise reproduction. This pertains to egg production, hatching rates and offspring health, and thereby the economic and environmental sustainability of insect farming. Studies varying the nutrient of interest, while providing adequate levels of other nutrients, to determine the optimal level of the target parameter can be used to establish the nutritional requirements of adults. In doing this, the paramount importance of larval nutrition on adult reproductive fitness should be acknowledged (Laursen *et al.*, 2024). Moreover, providing an optimal diet to the breeding population can hamper genetic selection for traits such as larval growth and bioconversion efficiency on effective barely suitable side-streams due to genotype-by-diet interactions (Sandrock and others, (In Press)).

Growth requirements

Over 96% of the feed utilised in insect farms is consumed during the larval or nymphal stages. Hence, meeting the nutritional requirements during the growth phase considerably impacts development time, composition, insect health and welfare, feed costs, environmental impact, and ultimately farm profitability.

One method that can be used to determine the net nutritional requirements for growth of a given nutrient, is the comparative slaughter technique (Chung and Baker, 1992). Here, the dietary level of the nutrient under study is provided at varying levels, with sufficient levels for other nutrients during a specific growth period. By comparing concentrations in animals sampled at the onset of such a trial, with concentrations of animals sampled at the end of the trial. The amount of that nutrient retained per day can then be quantified, and by dividing it by the body weight gained, the amount needed for that growth can be estimated

Generally, determining maintenance and growth requirements together is more practical. Quantification of required nutrients to optimise productivity at each stage of growth can be done via a factorial study, varying the level of the nutrient in question and harvesting at different time points to evaluate the dietary treatments. A growth trial can then be performed for the period of interest. The overall requirements then correspond to the lowest dietary concentration that maximises the target trait (for instance growth, or number of eggs) during the tested period. Once the levels of macro-nutrients are identified, detailed investigations can identify: (i) the first limiting amino acids in each species and their appropriate, eventually stage-specific, level in the diet (Koethe *et al.*, 2022; Sandrock, 2024; Spranghers *et al.*, 2024); (ii) effects and types of fibre in the diet; and (iii) requirement levels of minerals and micronutrients (Frooninckx, 2023; McFarlane, 1991). When doing such studies, potential interactions between nutrients should be taken into account (McFarlane, 1974).

Nutrient depletion/replacement studies and Dose-Response Studies

Nutrient depletion/replacement studies can identify whether a dietary constituent (e.g. an amino acid) is essential or not. The principle is to feed diets that are identical except for the inclusion of one investigated constituent, and asses its importance based on a specific read-out parameter (e.g. growth, survival, etc.). It is specific for the diet used under the tested conditions. Then, using dose-response studies, the requirement level of the essential nutrient for optimal performance can be determined. This approach works best using purified ingredients, with a known nutrient content, so that only the targeted nutrient content varies over treatments. For instance, sugar (i.e. sucrose), starch, protein concentrates (e.g. casein, peptone, or albumin), synthetic amino acids, fatty acids, vitamins, minerals, or sterols. For studies with larvae that live in their feeding substrate, providing a suitable structure of the diet is important. Semi-artificial diets, for instance wheat bran for mealworms or the Gainesville diet for Dipteran larvae (Hogsette, 1992) can be supplemented with purified ingredients (Barragan-Fonseca *et al.*, 2021; Clark *et al.*, 2015; Spranghers *et al.*, 2024).

When using starch as an ingredient, it must be digestible (non-resistant), but during diet preparation potential alterations of its structure and viscosity should be taken into account. For instance, boiling can lead to gelatinisation, which can affect free larval movement. Cellulose can be added as a bulking agent, and to provide volume for burrowing. Other physical properties, such as water holding capacity should also be taken into account, especially for semi-solid dipteran diets, as it affects their structure which can alter larval performance (Yakti *et al.*, 2023a).

Agar can be used to provide moisture *ad libitum* to mealworms and can be enriched with micronutrients (required in very low amounts) to ensure consumption, and avoid self-selection (see below) (Barragan-Fonseca *et al.*, 2021; Spranghers *et al.*, 2024).

When conducting studies beyond protein and energy requirements, isoenergetic and isonitrogenous diets should be used. This can be attained by including cellulose, starch or sugar, or bulk amino acids to replace other compounds. For example, when testing varying concentrations of the essential amino acid lysine, non-essential glutamic acid can be used as a substitute. In this example, both the nitrogen content and the molecular weight of the amino acids should be accounted for.

Methods to assess the nutritional value of feed ingredients and complete diets

Nutrient and energy digestibility of ingredients can vary greatly, and can be approached in different ways. For instance, apparent digestibility, initially developed in mammals, is defined as the difference between nutrients ingested and excreted through faeces, divided by ingested nutrients. The derived apparent digestibility coefficient, indicates how well the ingested nutrient is absorbed in the digestive system. Insects excrete a mixture of faeces and nitrogenous compounds (metabolic end-products such as uric acid), preventing direct assessment of apparent digestibility. Hence, the term 'approximate digestibility' is better fitting when using a mass balance approach based on ingested and excreted weights (Waldbauer, 1968). This approximation is suitable for species that feed on solid materials and produce solid excreta separable from residual feed, such as mealworms, crickets, and locusts. Frass can contain exuviae, which decreases the accuracy of the digestibility coefficient. Moreover, microbiota in the insect' intestinal tract can utilise or synthesise nutrients like amino acids, which hampers exact quantification of approximate digestibility values.

Moreover, endogenous secretions are excreted together with the microbiota and undigested dietary residues as part of the faeces. Hence, in pigs and poultry, digestibility is assessed at the ileum at the end of the small intestine. Ileal digestibility bypasses the impact of microbial activity on the accuracy of digestibility coefficients. Inaccuracies caused by endogenous secretions in the faeces can be overcome by quantifying these and correct digestibility coefficients to come to true digestibility coefficients. Methods for such advanced evaluations of digestibility are currently unavailable in insect studies. However, it is relevant to be aware that digestibility coefficients are confounded by microbial activity and endogenous secretions.

A few studies on yellow mealworms used the mass balance method to determine approximate digestibility coefficients. For instance, approximate digestibility for gross energy, dry matter, crude protein ash, ether extract, and acid detergent fibre was determined for a set of six raw materials for yellow mealworms (Pascual *et al.*, 2024). Similarly, approximate digestibility coefficients for ash, crude protein, and energy of wheat bran-based diets supplemented with fresh carrots and wet brewer's grains have been determined for yellow mealworms (Fasce *et al.*, 2022). Also in yellow mealworms, the dry matter digestibility coefficient of complete diets made from by-products with three levels of starch and protein was determined (Montalbán *et al.*, 2022, 2023).

More studies on the digestive utilisation of raw materials in yellow mealworm larvae and standardised methods for determining approximate digestibility coefficients across different conditions are needed. Often, such studies use plastic boxes or trays as the experimental unit, with variable numbers of insects. Trials are then conducted 1.5-2 months post-eclosion, allowing a constant feeding behaviour (Fasce *et al.*, 2022). For species that live inside their feed, a robust and reproducible method to quantify feed intake and produced excreta is needed to avoid under-estimation of digestibility coefficients. One approach is a progressive daily inclusion of feed ingredients followed by a 2-day period without feeding (Pascual *et al.*, 2024). In this way, practically all offered feed is ingested by the larvae, and contamination of the collected excreta is limited. In the latter experiment with six ingredients, only 1-2% (barley and soybean meal) was not ingested, which could be manually separated from the excreta. Another approach is the use of a uric acid complementary assay as described by Van Broekhoven *et al.* (2015) where the uric acid content of pure faeces is determined, and used to determine the fraction of pure faeces in a feed and faeces mixture allowing subsequent calculations of approximate digestibility coefficients (Montalbán *et al.*, 2022, 2023). For this, additional replicates are set up and provided with a restricted amount of feed and the animals are weighed frequently until they reach a constant weight. It is then assumed that the remaining substrate consists of pure faeces. Alternatively, the insects are separated from their feed and placed in an empty container to collect pure faeces. A downside of these methods is that frequent weighing, or moving the animals to an empty container, might influence their feeding behaviour or physiology (for instance, gut motility). Moreover, it does not take the possibility of coprophagy into account.

Yet another method, used in Orthopterans, is taking a subsample of feed, weighing the feed provided, weighing unconsumed feed and the excreta, and subsequent drying of these fractions to determine feed intake and faeces excretion on a DM basis (Oonincx *et al.*, 2010; Yakti *et al.*, 2023b). While the effect might be minor, this method disregards DM losses due to respiration of the provided leaves (Loon, 2005).

For crickets and mealworms choice-feeding experiments, based on self-selection as described by Waldbauer and Friedman (1991), can be used. Remaining food can then be separated from excreta by sifting the remaining mixture. The difference between ingested feed and excreta has been named assimilated feed (Morales-Ramos *et al.*, 2020a; Morales-Ramos *et al.*, 2020b). This method requires a larger particle size of the feed than of the faeces, and disregards the option that feed was reduced in size, but not ingested, similar to bread crumbs.

For dipteran larvae, which live in and feed on a semi-solid substrate, a precise assessment of the amount of feed ingested is still to be developed and would require a way to separate excreta from non-ingested feed and exuviae in the frass. An alternative approach to calculate estimated digestibility considers the weights of distributed feed and frass. Complete ingestion of distributed feed can be assumed when working either with high larval density (Guillaume *et al.*, 2023), or an extended feeding time (Guillaume, 2024). The asymptotic estimated digestibility value can then serve as a proxy for the maximum collective digestive efficiency of larvae and surrounding microbiota. When using at least four treatments with either varying density or feeding time, and three of these treatments yield similar digestibility coefficients, this indicates an asymptotic value has been reached (Guillaume *et al.*, 2024, 2023). This method was developed for BSF larvae, but might also be suitable for other Diptera, such as the housefly.

Another method involves the use of indigestible markers in digestibility studies. This avoids both tedious ingesta quantification, and complete faeces collection. Approximate digestibility is calculated as the difference of concentration of an indigestible marker in excreta and in the feed, divided by the concentration in excreta. This only requires a sample of pure excreta. However, this method relies on analytical quantification procedures, which are costlier than a simple mass balance approach. A marker method is especially suitable in digestibility assays for species for which excreta can be easily collected, such as most Lepidoptera, for instance silkworms (*Bombyx moryi*), most Orthoptera, and quite possibly Coleoptera (Matsura and Matsuda, 1993; McGinnis and Kasting, 1964). This indirect method was recently tested on BSF larvae, with 1% (DM) chromic oxide as a marker, using specific adaptations for excreta sampling. After three days of feeding with marked substrate, larvae were rinsed and placed in an empty crate for 24 hours (Guillaume, 2024). Excreta were then collected by dilution in distilled water using a micropipette, and subsequently the water was evaporated. An alternative to 1% (DM) chromic oxide is with 0.5% dry matter titanium dioxide (Veldkamp, personal communication). Suitable markers remain inert in the digestive tract, and do not interfere with ingestion, digestion, or growth processes. These can be artificially incorporated in experimental diets, such as metal oxides or stable isotopes, or can be endogenous, such as lignin or silicon (Van Loon, 2017).

Choice/self-selection experiments

Choice experiments have been conducted on insects to understand various aspects of the ecology and physiology aspects including mating choices (Cease, 2013; Latchininsky *et al.*, 2016), olfactory clues (Dadd, 1961a), and nutritional preferences (Dadd, 1960a). In contrast to no-choice experiments in which insects are provided and "forced" to feed on one diet, choice experiments have been widely used to explore and better understand their nutritional requirements. The idea behind this approach is that there is a species-specific balance of nutrients that they aim to attain through their feed choices to optimise their growth and development. Different feed ingredients are simultaneously offered to insects in specially constructed multiple-choice arenas and the relative consumption of each ingredient is determined to calculate the chosen consumption ratio. This ability to select and consume different types of feed to regulate their nutrient intake has been defined as dietary self-selection (Behmer, 2009; Waldbauer and Friedman, 1991). Nutrient self-selection has been used for various edible insect species. A downside of this approach for investigating insects' nutritional requirements is that insects' attraction in general can be governed by various factors, for instance, sensory ques (Cease, 2013). These should be critically considered when interpreting the observed effects (Dadd, 1961a). For instance, the particle size of the diet may affect the preference and choice of crickets, as they tend to reject large particles despite the fact that they may contain essential nutrients (Van Peer *et al.*, 2024). This indicates that the provided ingredients in choice experiments should be of small and similar particle size. Similarly, the attractiveness of a diet and subsequently the insect feeding choices in self-selection experiments may also be influenced, apart from the diet nutritional content and value, by its texture (Cohen, 2003). Therefore, special care should be taken when conducting choice experiments to minimise the impact of other parameters that may affect the attractiveness of the tested ingredients and diets, in order to be able to attribute the observed results solely to their nutritional value.

Dietary self-selection has been used in yellow mealworm larvae to determine digestible protein and carbohydrate intake. For instance, Morales-Ramos *et al.* (2011) evaluated four diets composed of two components, i.e., wheat bran and dry potato flakes, at different ratios (9:1, 8:2, 7:3 and 6:4) in a no-choice and a two-choice experiment, with the 8:2 self-selected ratio resulting in the best larval development. In a similar follow-up study, six feed ingredients, i.e., dry potato flour, dry egg white, soy protein, peanut oil, canola oil and salmon oil, were offered at different ratios in a self-selection experiment to yellow mealworm larvae to produce a dietary supplement (Morales-Ramos *et al.*, 2013). A similar study with yellow mealworms offered various food products and agricultural by-products and quantified the optimal macronutrient ratios for proteins (23%), lipids (6%), and carbohydrates (71%) (Morales-Ramos *et al.*, 2020b). In a multiple-choice arena with pelleted feed, highly similar optimal levels (protein (20–23%), lipids (9-10%), and carbohydrates (67–72%)) were suggested (Kröncke and Benning, 2022). In the latter study, nutrient self-selection was also used to reveal age-specific differences in nutritional requirements, that younger larvae (6 weeks) prefer more carbohydrates for growth than older ones (10 weeks) while protein and lipid intake increase within this period, possibly to prepare for the energy demanding metamorphosis. While less commonly studied, self-selection studies can also be applied for adult nutrition (Rho and Lee, 2014, 2016, 2022).

Feeding choices of locusts are complex behavioural processes, influenced by swarming and mass cohesion (Cease, 2013; Latchininsky *et al.*, 2016), olfactory clues (Dadd, 1961a) and visual memory (Dadd, 1960a,b) amongst other factors. Such experiments can significantly contribute to understand their nutritional needs. For instance, the feeding choices of migratory locusts subjected to diets deficient in available carbohydrates, protein, or both, or a control diet and subsequently allowed to self-select, selected for the nutrient they lacked (Simpson *et al.*, 1988). Besides selecting for macro-nutrients, migratory locusts nymphs can also regulate salt intake by self-selection (Trumper and Simpson, 1993). Similarly, house cricket self-regulate intake of macro and micro-nutrients, elucidating their nutrient requirements (Morales-Ramos *et al.*, 2020a).

Indirect calorimetry

Indirect calorimetry is used to determine energy expenditure and substrate utilisation. Two important metrics in the context of edible insects are the Specific Metabolic Rate (SMR) and the Respiratory Exchange Ratio (RER), also called the Respiratory Quotient (RQ). Specific metabolic rate is a physiological concept that quantifies energy expenditure per mass unit, typically expressed in watts per kilogram (W/kg). It helps understanding how different organisms utilise energy and how their metabolic processes are influenced by factors such as body size, environmental conditions, and physiological state. Heat production can be determined with a calorimeter allowing calculation of the SMR with the following equation:

$$\text{SMR} = \frac{-(\text{heat released from asample} - \text{heat released from blank})}{\text{Weight of larvae}}$$

In BSFL the SMR decreases from approx. 12 µW/mg in the 3rd instar to 2 µW/mg in the non-feeding 7th instar (Gligorescu *et al.*, 2019).

The RER can be calculated based on the ratio of CO_2 produced to O_2 consumed and indicates which macronutrient (carbohydrate, protein, and fat) is predominantly utilised for energy (Brouwer, 1965) using the following equation:

$$\frac{\text{RER}}{\text{RQ}} = \frac{V_{CO_2}}{V_{O_2}}$$

An RER of 1 indicates an organism uses carbohydrates as energy source, for fats this is approx. 0.7, and for proteins it is 0.9. An RER above 1.0 indicates CO_2 is produced without oxygen consumption, for instance due to anaerobic fermentation or *de novo* synthesis of fatty acids from carbohydrates (Gerrits *et al.*, 2015). While informative in controlled experimental settings, RER values should be interpreted with caution in practical settings. As organisms would utilise multiple nutrient types simultaneously if available, the calculated RER, is an average pertaining to the nutrients in concert. Moreover, CO_2 and O_2 measurements include the metabolic activity of the insects, but also their associated microbiome and the microbiome in the substrate. Hence, the RER can be used to better understand the role of microbes in nutrient conversion. The RER can also provide insight in optimising dietary formulations for promoting or limiting fat storage, thereby influencing growth, production efficiency, and insect composition. applied This method has been applied in BSFL (Parodi *et al.*, 2020) and in the locust *Schistocerca cancellata* (Serville); Orthoptera: Acrididae) (Talal *et al.*, 2021).

Additional information can originate from also measuring NH_4, N_2O and CH_4 (Coudron *et al.*, 2024; Parodi *et al.*, 2021). This information is valuable as it considers gaseous metabolic products, ignored in other methods such as raw material digestibility indicators, which combines estimates of nutrient assimilation, thermogenesis, energetics of physical activity and GHG emissions. Moreover, indirect calorimetry is typically non-destructive and can produce results in second resolution. It is a relatively rare in edible insect research and considered challenging as it requires a well-built respiratory chamber, an accurate data collection setup, and a suitable data analysis pipeline (Gerrits *et al.*, 2015, 2019; Parodi *et al.*, 2020; Parodi *et al.*, 2021). For insects that live in their feeding substrate, the contribution of substrate and gut microbiome to gaseous emissions is difficult to determine, and larval handling (transferring them from their substrate to a respiratory chamber) can alter larval activity, leading to the generation of non-representative data.

Metabolic studies (*e.g. stable isotope tracing*)

Most elements of biological interest (e.g., carbon, nitrogen, hydrogen) have several isotopes, with the lighter form generally present in greater abundance (for example abundance of ^{12}C in terrestrial ecosystems is 98.9% and for ^{13}C this is 1.1%; for ^{14}N this is 99.6% and for ^{15}N it is 0.4%) (Criss, 1999). Relative natural abundances of isotope forms, usually expressed as (δ) delta values as parts per thousand, are usually unique to organisms and ecosystems. In fact, the isotopic composition of organisms can be used as a "signature" to understand nutrient flows in various ecological studies (see Quinby *et al.* (2020) for an overview of insect-related studies) as it reflects the isotopic composition of their diets.

Besides naturally-occurring isotopes, artificially-enriched stable isotopes can be added as tracers to assess the digestion and metabolism of a compound of interest. For instance L-arginine consisting of ^{15}N instead of ^{14}N, or glucose consisting of ^{13}C instead of ^{12}C) are chemically and functionally identical to their naturally occurring counterpart (the tracee), but can be detected and tracked in body tissues, excreta, or respired air due to their higher atomic mass of ^{15}N or ^{13}C. Isotopic tracers allow quantification of nutrient absorption, body stores, fluxes and elimination, and can thereby indicate nutrient metabolism of humans and livestock alike (Gerrits and Labussière, 2023; Schoeller, 2002). There are multiple metrics to quantify isotopic enrichment when using tracers (Table 1). This method does require knowledge on the background isotopic abundance (without enrichment).

While stable isotopes have been used to assess the dietary ecology of insects relevant for agriculture (e.g., pests, pollinators) (Snart *et al.*, 2015), in nutritional studies on edible insects it is still scarce. Recently, Parodi *et al.* (2022) used the stable isotope ^{15}N in NH_3 to explore whether this N can be incorporated into BSFL body mass during pig manure bioconversion and found that at least 13% of pig manure NH3-N can be incorporated into BSFL body mass.

TABLE 1 Common metrics to express isotopic enrichment

Unit	Description	Example
Tracer-to-tracee ratio (TTR)	Amount of tracer relative to to the amount of tracee.	A TTR of 0.05 indicates that the amount of tracer is 5% of the amount of tracee.
Mole percent (mol%) or atom percent (at %)	Molar ratio of the tracer molecule or atom and the sum of the tracer plus the tracee molecule or atom.	A mol% or at% of 0.05 indicates that the amount of tracer is 5% of the amount of tracer plus tracee.
Mole percent excess (MPE, mol%) or atom percent excess (APE, at%)	Similar to mol% and at% but subtracting the natural isotopic abundance.	A MPE mol% or APE at% of 0.05 indicates the amount enriched molecule or atom is exceeded by 5% compared to the natural isotopic abundance of the same molecule or atom (without tracer)
Delta value (δ) relative to an international standard (PDB)	Delta values represent the ratio between a heavy and a lighter isotope (e.g., $^{13}C/^{12}C$) but expressed in parts per thousand (‰). A PDB consists of the delta value of a sample relative to an international standard. International standards have high delta values, and therefore most PDB are negative.	If the $^{13}C/^{12}C$ ratio of a sample is 0.010743 and the $^{13}C/^{12}C$ ratio of the international standard is 0.112372, the delta value relative to the international standard would be –44‰ ((0.010743–0.112372)/0.112372) * 1000

Hence, this method offers great potential to better understand the metabolic fate of dietary components.

In summary, indirect calorimetry allows estimation of macronutrient (e.g., fats, carbohydrates) oxidation, while isotopic studies allows quantification of the oxidation of specific components (e.g., a certain fatty acid, or monomer) by measuring rates of $^{13}CO_2$ expiration. In combination with the measurement of ^{13}C in body stores and excreta, these methods can help reconstruct the fate of specific components.

5 Conclusion and future outlook

Insect farming is rapidly growing as an approach for sustainable protein production for food and feed applications, addressing global food security challenges and contributing to circularity in food production systems. Advancing knowledge of the nutrient requirements of farmed insect species will help improve their performance and enhance farming productivity by developing precise and cost-effective feed formulations that meet their dietary needs. This chapter summarises the current knowledge on the nutrient requirements for the most commonly produced insects used as food or feed. It provides a synthesis of the in-depth studies conducted during the fifties, sixties and seventies of the last century, with the most recent studies on the topic of nutrient requirements. Furthermore, it identifies both gaps in knowledge, such as micronutrient requirements, specific amino acid requirements, the interaction between diet and microbiota, and between diets and genotypes, as well as ways to fill these gaps. Moreover, it describes various methodologies hitherto used to determine these requirements and directions for further method development. As the field of edible insect nutrition is rapidly developing, it is to be expected that interdisciplinary studies will contribute to the expansion of knowledge on nutrient requirements and the further development and application of suitable experimental methods. This will lead to the creation of feed evaluation systems, and consequently the formulation of efficient diets for optimal edible insect production. Valorisation of these elements will contribute to sector development and allow effective use of insects to increase the circularity of our food system.

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