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REVIEW

Health control of insects used as feed materials and experimental models: state of the art, prospects for future with utilization of biochemical assays – a review

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ABSTRACT. The feedstock market is changing in response to the increasing demand for lipid and protein sources, alongside the pursuit of more sustainable agriculture. As a result, alternative nutrient sources are being extensively studied. Insects have gained attention as a promising feed material due to their high protein digestibility, favourable amino acid and lipid profiles, as well as nutraceutical potential. However, studies on their utilisation often neglect the health status of insects, which may influence feed quality and the health of animals consuming it. This review examines the potential of insects as a feed material considering their fat and protein content, amino acid profiles, potential application in bioconversion, controversies surrounding their consumption, regulatory frameworks, and the potential of synanthropic species as bioindicators. Regarding the lack of standardised methods for monitoring the health and welfare of these 'small livestock' and experimental models, we assessed the applicability of biochemical assays commonly employed in toxicological studies. Based on our evaluation, we identified and selected most suitable parameters for the purpose under discussion.

Introduction

For several decades, the demand for feed materials, particularly protein and fat sources, has steadily increased across various sectors of livestock production, especially in areas where production has become more intensive. Historically, both animal-derived meals and plant-based materials, as well as by-products from the food and agricultural industries, have been used to meet this demand. However, modern challenges related to sustainable development, including water-efficient management, reduction of greenhouse gas emissions, use of by products like crop residues, climate change and biodiversity protection require optimalisation of animal husbandry practices. Over the past few decades, the growing demand for alternative protein and lipid feed materials has prompted the experiments on the use of insects and substances they synthesise in feed products (Table 1). The number of publications on this topic has been steadily increasing over this period (Kierończyk et al., 2022). Studies investigating the utilisation of insect-derived feed materials in animal nutrition have demonstrated their unique nutritional properties. These feed components have high protein digestibility and a favourable amino acid profile, meeting the nutritional requirements of various animal species. Additionally, certain insect species are characterised by a low fat content, while providing a high concentration of polyunsaturated fatty acids. Moreover, insect-based feeds demonstrate nutraceutical potential, offering functional benefits that may enhance animal health and performance (Navarro del Hierro et al., 2020; Kierończyk et al., 2022, 2024; Tables 2 and 3). However, the chemical makeup of insects is strongly influenced by the composition of their rearing substrate. Changes in diet not only affect the protein and fat content or dry matter of insects, but also alter their profile of fatty acids and amino acids, as well as the content of micro- and macronutrients (Jajić et al., 2022; Table 2). Therefore, understanding the impact of insect diets on their body composition is essential for developing targeted feed formulations that meet livestock nutritional needs. In addition, insect meal has the potential to become a more environmentally sustainable feed source compared to the traditionally used fish meal or plant-based alternatives, such as soybeans. This is largely due to the possibility of rearing insects on bio-waste and agro-residues, enabling the closure of matter cycles within food production systems (van Huis, 2020; Beyers et al., 2023). However, current regulations prohibit insect producers from using agri-food waste if the end products are intended for human consumption (European Commission, 2017a,b; Lähteenmäki-Uutela et al., 2021). Additionally, even intensive insect farming exerts lower pressure on biodiversity, as it requires less land area compared to conventional livestock farming and can be integrated into existing infrastructure.

Extensive research has been devoted to exploring the potential of insects, commonly used by vivarium hobbyists as feed for companion animals, in food and animal feed applications. These include yellow mealworms (*Tenebrio molitor*), superworms (*Zophobas morio*), various species of crickets and locusts (order Orthoptera), black soldier fly larvae (*Hermetia illucens*), cockroaches/roaches (order Blattodea), and the African palm weevil (*Rhynchophorus phoenicis*). Some of these species, such as the desert locust (*Schistocerca gregaria*), Jamaican field cricket (*Gryllus assimilis*) or Turkestan cockroach (*Blatta lateralis*) have a high protein and low body fat contents (Hong et al., 2020; Tables 1 and 2).

Table 1. Selected insect species used in animal nutrition worldwide

Species	Use of insects or substances derived from insects	Application as a lipid and/or protein source	Fed species	References
Black soldier fly (*Hermetia illucens*)	insects – mostly larvae	lipid and protein source	dog (*Canis familiaris*)	Freel et al. (2021)
			rainbow trout (*Oncorhynchus mykiss*) african sharptooth catfish (*Clarias gariepinus*)	Bartucz et al. (2023)
			broiler chicken (*Gallus gallus domesticus*)	Kierończyk et al. (2024)
Desert locust (*Schistocerca gregaria*)	insects	lipid and protein source	nile tilapia (*Oreochromis niloticus*)	Ramzy et al. (2022)
			broiler chicken (*Gallus gallus domesticus*)	Al-Homidan et al. (2024)
Honey bee (*Apis mellifera*)	royal jelly	lipid source	zebrafish (*Danio rerio*)	Vural et al. (2023)
House cricket (*Acheta domestica*)	insects	lipid and protein source	rainbow trout (*Oncorhynchus mykiss*)	Turek et al. (2020)
Silkworm (*Bombyx mori*)	chrysalis (spent silkworm pupae)	lipid and protein source	chicken (*Gallus gallus domesticus*)	Miah et al. (2020)
Superworm (*Zophobas morio*)	insects – larvae	lipid and protein source	rainbow trout (*Oncorhynchus mykiss*)	Turek et al. (2020)
			grower native chicken (*Gallus gallus domesticus* L.)	Magsalay et al. (2024)
Termites (*Macrotermes bellicosus*)	insects	lipid and protein source	broiler chicken (*Gallus gallus domesticus*)	Mali et al. (2020)
Termites (*Macrotermes falciger*)	insects	lipid and protein source	mozambique tilapia (*Oreochromis mossambicus*)	Nephale et al. (2024)
Yellow mealworm (*Tenebrio molitor*)	insects – larvae and imago	lipid and protein source	broiler chicken (*Gallus gallus domesticus*)	Biasato et al. (2016)
			blackspot sea bream (*Pagellus bogaraveo*)	Iaconisi et al. (2017)
			broiler chicken (*Gallus gallus domesticus*), pig (*Sus domesticus*)	Hong et al. (2020)
			rainbow trout (*Oncorhynchus mykiss*)	Jeong et al. (2020)

The European Union (EU) is promoting the use of insect meal as a promising feed material for the future. In July 2017, the EU began creating legislation and permits for the use of insects as processed animal protein (PAP) from insect. This legislative amendment allows the transformation of insects into insect meal (dried insect ground to meal), which is high in energy and protein content (Adli, 2021)

Table 2. Protein and fat content in the body of selected insects used in animal nutrition

Species	Crude protein content, g/100 g dry weight	Crude fat content, g/100 g dry weight	References
African palm weevil	27.50 ± 1.51	71.62 ± 7.61	Mba et al. (2017)
(*Rhynchophorus phoenicis*) larvae*	31.05 ± 0.55	65.35 ± 0.14	Anankware et al. (2021)
Black soldier fly	40.4 ± 1.8	33.5 ± 3.0	Józefiak et al. (2018)
(*Hermetia illucens*) larvae	42.0 ± 0.1	36.2 ± 0.2	Huang et al. (2019)
	28 ± 1.4	33.7 ± 0.28	Matthäus et al. (2019)
Desert locust	76 ± 0.9	12,97 ± 0.7	Zielińska et al. (2015)
(*Schistocerca gregaria*)	35.3 ± 2.6	13.3 ± 2.0	Haber et al. (2019)
	45.38 ± 0.3**	12.03 ± 0.2*	Mmbone et al. (2023)
Dubia roach	63.0	21.4	Kulma et al. (2016)
(*Blaptica dubia*) imago	52.87 ± 1.01^{f}	35.49 ± 3.68^{f}	Lam et al. (2021)
	54.32 ± 2.0^{m}	35.51 ± 0.71^{m}	
	96.9	13.3	Cerreta et al. (2022)
Jamaican field cricket	56.4 ± 2.5	23.8 ± 2.1	Józefiak et al. (2018)
(*Gryllus assimilis*) imago	55.6 ± 1.1	11.9 ± 0.5	Mlček et al. (2018)
	45.75 ± 2.25	20.0 ± 1.94	Quinteros et al. (2022)
Superworm	39.0 ± 1.0	39.0 ± 4.0	Adámková et al. (2016)
(*Zophobas morio*) larvae	47.4 ± 2.2	36 ± 3.4	Kulma et al. (2020)
	46.8	41.7	Prachom et al. (2021)
Turkestan cockroach	58.4	14.5	Kulma et al. (2016)
(*Blatta lateralis*) imago	54.6 ± 2.5	26.1 ± 2.3	Józefiak et al. (2018)
	77.1	17.6	Cerreta et al. (2022)
Yellow mealworm	56.3 ± 2,5	25.3 ± 2.0	Józefiak et al. (2018)
(*Tenebrio molitor*) larvae	45.6 ± 2.8	34.5 ± 3.2	Costa et al. (2020)
	38.9 ± 0.2*	45.2 ± 0.4*	Jajić et al. (2022)
	71.2 ± 1.1*	6.1 ± 1.2*	

* – for more data see Siddiqui et al., 2024; ** – depending on diet; m/f – depending on sex (m – male, f – female)

Current research on the application of insects and insect-derived products primarily focuses on evaluating their impact on the health status of livestock fed insect meal-enriched feed. However, limited attention has been given to assessing the health condition of the insects utilised for meal production. Investigating whether the health status of insects intended for consumption influences the quality of the resulting insect meal, and whether this could subsequently impact the health and welfare of animals consuming such feed, would provide critical insights. Diagnostic methods for this purpose could be developed based on knowledge gained from toxicity tests on insects. These methods could incorporate biochemical assays, whose utility has been evaluated in the present study. Furthermore, such diagnostic tools could be employed in toxicity testing or in environmental monitoring programmes utilising the health status of insect bioindicator species.

Material and methods

To compile the bibliography, a systematic search was conducted using the Google Scholar database in July 2024, according to the PRISMA2020 guidelines described by Page et al. (2021). The review focused on scientific publications in English using the following keywords: ‘edible insects’, ‘edible insects as feed replacement’, ‘insects as feed replacement’, ‘safety of edible insects’, ‘edible insect microbiota’, ‘potential insect bioindicators’, ‘insect bioindicators’, ‘edible insect regulations’ and ‘insects and accumulation of heavy metals’. These publications were organised into the following sections: Introduction, Safety regulations of insect utilisation as food and feed materials, Controversies surrounding edible insects and potential contamination, Insects in waste bioconversion, and Potential use of synanthropic insects as bioindicators. Priority was given to studies published within the last decade. Additionally, European Union regulations concerning the use of insects as feed materials and food were reviewed. For sections: Insect health assessment in diagnostics and toxicological studies, and Biochemical assays applied in insects and significance of measured parameters in insect health assessment, a search was conducted for scientific publications in English using keywords

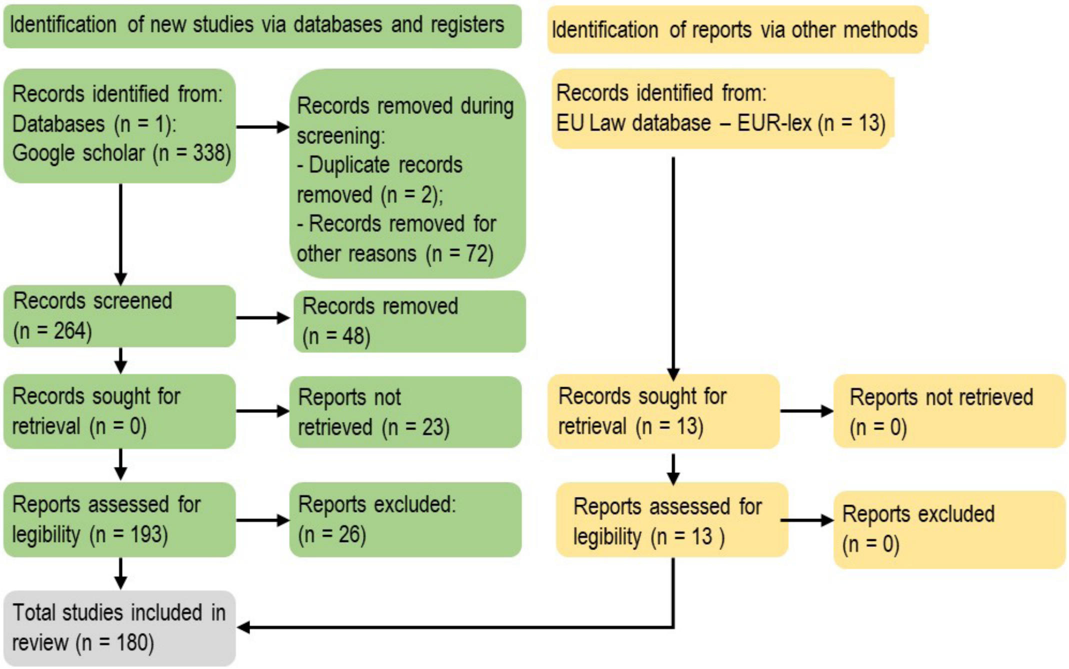

Figure 1. Research and screening process used to qualify manuscripts according to the PRISMA2020 guidelines (Page et al., 2021)

such as ‘insect’, ‘toxicology’, ‘pest control’, ‘ecotoxicology’, ‘insect diagnostics’ and ‘biochemical assays’. Following the identification of commonly used biochemical assays, further searches for specific parameters were conducted by combining the keywords ‘parameter name’ + ‘insect’ to understand its role in invertebrates. Additional searches with ‘parameter name’ + ‘vertebrate’, ‘fish’, and livestock species such as ‘cow’, ‘poultry’, ‘swine’ were carried out to compare the role of these parameters and identify potential differences between insects and vertebrates. Publications from the past two decades were prioritised, though older publications were also included after a rigorous evaluation of their relevance and validity of knowledge. These methods resulted in the retrieval of 338 reports from the Google Scholar database and 13 reports from the European Union law database (EUR-lex). Subsequently, the selection process was applied, as illustrated on Figure 1. Of 338 initially selected reports, 74 articles were excluded due to duplications or insufficient relevance to the keywords of interest. A further selection process involved reviewing 264 articles, resulting in the exclusion of 71–48 for inadequate language translation and 23 for insufficient information. Out of the 193 research publications chosen for the final analysis, 26 were later excluded during the writing process, as they contained the same knowledge as more recent articles. Ultimately, a total of 167 research articles and 13 European Union law reports were included in this review (Figure 1).

The essential amino acid indices (EAAi) in Table 3 were calculated using the adjusted EAAi formula of Peñaflorida (1989), employing the following equation:

$$\mathrm{EAAi} = \sqrt[n]{\frac{\mathrm{ea}_1}{\mathrm{EA}_1} \times \frac{\mathrm{ea}_2}{\mathrm{EA}_2} \times \ldots \times \frac{\mathrm{ea}_n}{\mathrm{EA}_n}},$$

where: ea – essential amino acid in insect (g/kg protein); EA – essential amino acid in the reference – chicken egg white (g/kg of protein); n – number of essential amino acids for humans.

Safety regulations of insect utilisation as food and feed materials

Products containing insect protein raise many controversies, concerns and prejudices, especially in Europe, Canada and the United States of America (USA), where there is no tradition of eating these animals, unlike in Asia, Central America, South America and Africa (Hartmann et al., 2015; Bessa et al., 2020; van Huis, 2020). A major point of contention involves concerns about their potential health effects. Specifically, issues have been raised regarding their allergenic properties and the limited knowledge surrounding

Table 3. Amino acid content in the bodies of selected insects used in animal nutrition compared to chicken egg white (conventional protein feed material); fish meal and plant derived alternative protein feed materials – soybean meal and white lupin seed meal

Amino acid, g/kg of protein	Black soldier fly (*Hermetia illucens*) larvae	Desert locust (*Schistocerca gregaria*)	Dubia roach (*Blaptica dubia*)	Jamaican field cricket (*Gryllus assimilis*)	Superworm (*Zophobas morio*) larvae	Turkestan cockroach (*Blatta lateralis*)	Yellow mealworm (*Tenebrio molitor*)	Chicken egg white	Fish meal	Soybean meal (*Glycine max*)	White lupin seed meal *(Lupinus albus*)
VAL*	57.4–61.6	30.9–57.47	66.3–73.8	40.3–46.7	29.0–67.4	12.3–81.3	47.6–64.6	58.9 ± 5.8	36.95–54.3	2.27–25.68	1.06–4.1
TRP*	13.2± 0.4	–	–	7.5–25.7	4.8 ± 1.1	1.66	15.0 ± 0.1	9.0 ± 1.4	8.29–11.8	7.49	0.5–0.7
ILE*	45.8–53	20.9–28.91	37.2–41.4	13.9–34.2	24.3–49.7	7.73–101.3	31.0 –50.1	61.3 ± 4.6	32.42–47.4	2.18–24.61	1.26–4.3
LEU*	73.8–89.2	35.3–78.95	64.6–71.5	42.6–66.3	53.0–76.9	9.7–12.0	73.5 –83.5	77.4 ± 6.4	52.78–77.4	3.75–41.20	1.92–7.8
PHE*	51.2–64.4	14.5–19.03	31.9–37.8	5.2–34.0	18.4–49.8	7.67–46.7	36.4	51.7 ± 4.7	28.65–41.2	2.48–27.29	1.02–5.6
HIS*	38.3–43.6	11.1–20.95	28.4–34.8	9.5–25.2	15.9–39.0	5.49–47.7	27.7–36.1	18.4 ± 2.1	16.59–24.1	1.29–14.45	0.51–3.3
LYS*	63.0–69.3	24.9–35.92	53.8–62.8	52.8–85.4	30.4–53.1	12.8–94.0	24.8–62.2	59.5 ± 5.9	56.55–79.1	2.88–33.71	1.19–4.9
ARG*	50.1–53.7	25.5–40.54	56.1–59.0	16.6–41.8	26.3–58.1	14.0–89.8	43.5–55.9	49.0 ± 3.9	43.73–57.0	39.06	11.1–11.4
MET*	19.9–27.4	7.88–9.4	13.6–16.3	4.3–23.3	6.2–16.7	3.35–7.8	15.2 ± 0.4	30.2 ± 2.7	21.11–30.2	0.70–7.49	0.3–0.7
THR*	43.3–46.4	18.0–36.19	16.5–43.1	30.2–41.8	20.2–43.1	7.89–23.8	44.9 –56.8	41.2 ± 3.7	30.91–43.7	1.91–20.33	0.83–3.5
TYR	65.2–77.9	21.6–33.52	46.9–80.6	42.3–61.0	37.3–95.8	14.3–59.9	57.9	34.7 ± 3.1	21.87–33.3	18.73	1.5–2.0
CYS	9.5–14.5	3.4–9.5	1.7–12.9	6.0–11.6	3.7–12.6	0.9–1.44	11.3 ± 0.1	20.4 ± 1.9	6.03–9.4	8.56	1.8–2.1
ASP	104.4–110.4	34.7–66.92	28.0–99.9	80.0–92.7	41.1–83.7	15.1–39.3	91.8 –101.0	94.9 ± 7.7	65.60	60.99	9.9–11.1
SER	39.2–45.5	17.5–34.15	17.7–47.5	4.1–39.0	26.3–48.8	8.38–27.9	50.2 –59.1	60.3 ± 4.1	30.16	24.61	4.1–4.9
GLU	107.6–136.9	51.3–109.37	48.0–131.0	22.7–106.2	55.2–135.7	22.6–72.2	121.2–130.0	118.6 ± 8.9	95.00	95.77	22.9–24.2
PRO	46.1–59.7	30.4–67.84	39.3–68.4	5.3–52.0	34.0–60.6	10.6–45.7	77.8–98.6	34.7 ± 3.1	28.65	26.75	3.1–3.8
GLY	42.6–49.7	25.9–49.82	64.6–166.2	39.1–44.9	27.7–56.2	12.4–128.6	49.5–68.2	29.8 ± 2.7	44.49	22.47	4.3–4.4
ALA	56.3–63.1	70.3–80.46	88.0–108.9	33.9–63.6	43.1–73.0	16.7–118.4	72.4–103.4	51.5 ± 4.7	45.99	23.01	3.1–3.2
EAAi	1.04–1.20	0.41–0.67	0.78–0.97	0.40–0.99	0.46–0.97	0.18–0.76	0.82–1.07	–	0.74–1.05	0.08–0.53	0.03–0.09

VAL – valine, TRP – tryptophan, TRP – tryptophan, ILE – isoleucine, LEU – leucine , PHE – phenylalanine, HIS – histidine, LYS – lysine, ARG – arginine, MET – methionine, THR – threonine, TYR – tyrosine, CYS – cysteine, ASP – aspartate, SER – serine, GLU – glutamate, PRO – proline, GLY – glycine, ALA – alanine; EAAi – essential amino acid index; calculated from above values, according to adjusted methodology described by Peñaflorida (1989); * – essential amino acids (for humans), values were obtained: Kulma et al. (2016), Perez-Santaescolastica et al. (2023) for Dubia roach; Janssen et al. (2017), Huang et al. (2019) for Black soldier fly larvae; Janssen et al. (2017), Costa et al. (2020) for yellow mealworm, Finke (2013), Kulma et al. (2016) for Turkestan cockroach; Bednářová et al. (2014), Perez-Santaescolastica et al. (2023) for superworm; Bednářová et al. (2014), Oibiokpa et al. (2018) for Jamaican field cricket; Zielińska et al. (2015), Abd-El Wahed and Ahmad (2019) for desert locust; Vrabec et al. (2015), Szczepański et al. (2022) for soybean meal; Sujak et al. (2006), Szczepański et al. (2022) for white lupin meal; Cho and Kim (2011), Vrabec et al. (2015) for fish meal; Bednářová et al. (2014) for chicken egg white the existence and implementation of standardised guidelines or regulations to ensure the health and safety of cultured insects. These uncertainties underscore the need for further research and the dissemination of knowledge concerning regulatory frameworks to comprehensively address these issues (van Huis, 2020; Wangberg et al., 2021). Under European Union (EU) legislation, insects are classified as novel food (European Parliament, 2015). Specific insect species authorised for sale as food include yellow mealworm larvae, migratory locust (*Locusta migratoria*), lesser mealworm larvae (*Alphitobius diaperinus*) and domestic cricket (*Acheta domesticus*) (EFSA Panel on Nutrition et al., 2021a,b; European Commission, 2023a,b). Import of insects for human consumption is permitted exclusively from designated countries, namely Canada, South Korea, Thailand, Switzerland, the United Kingdom, and Vietnam, provided that production standards and safety criteria comply with EU regulatory requirements (European Commission, 2021a). European Union legislation specifies the insect species approved for farming, which include the common house fly (*Musca domestica*), yellow mealworm, lesser mealworm, domestic cricket, tropical house cricket (*Gryllodes sigillatus*), Jamaican field cricket, and silkworm (*Bombyx mori*). Additionally, the law defines acceptable substrates for insect rearing, explicitly prohibiting the use of slaughter by-products, disposal products, manure, and catering waste as feed substrates (European Commission, 2017a,b, 2021b). The legislation further imposes restrictions on the levels of harmful substances in insect feed products, aligning these requirements with safety standards established for other animal feed types (European Parliament, 2002a). Furthermore, insects intended for use as food products must comply with the same hygiene and safety standards as conventional food products. These include adherence to established protocols for contamination control, quality assurance, and consumer health protection to ensure their suitability for human consumption (European Parliament, 2002b, 2004, 2005). In Canada, insects are similarly classified as novel foods under the regulatory framework and are subject to a mandatory registration and assessment process prior to being authorized for marketing. Additionally, the use of insects in animal feed is strictly regulated. Currently, only black soldier fly larvae are approved for use as feed, and this authorisation is limited to aquaculture and poultry (Sogari et al., 2019; Lähteenmäki-Uutela et al., 2021). In the USA, edible insects are classified as food additives under a regulatory framework that requires compliance with safety and labelling requirements. Only dried larvae and processed meal derived from black flies are approved for use as animal feed, and their use is restricted to salmonids, poultry and pigs. In contrast, in regions where insects have been traditionally consumed as a dietary ingredient, regulatory oversight of their use is often absent, with no formalised documentation or standards governing their production or consumption (Sogari et al., 2019; Lähteenmäki-Uutela et al., 2021).

Controversies surrounding edible insects and possible contamination

In Europe, Canada and the USA, the consumption of aquatic invertebrates is widely accepted and often regarded as uncontroversial, with certain species considered delicacies. This contrasts with the consumption of terrestrial invertebrates, including insects, which continues to face regulatory scrutiny and cultural resistance. A key factor contributing to this distinction is the preparation practices associated with aquatic invertebrates. In most cases, as with vertebrates, the digestive tracts of aquatic invertebrates are removed during processing, thereby eliminating excreta and associated microbiota from edible portions. Additionally, well-established culinary and safety protocols for the preparation of aquatic invertebrates further strengthen their acceptability for human consumption (Venugopal and Gopakumar, 2017). However, the removal of the digestive tract does not eliminate all accumulated xenobiotics, such as heavy metals or pesticides, which can accumulate in the tissues of both aquatic invertebrates and vertebrates. For this reason, the consumption of aquatic invertebrates is recommended to be rational and moderate (Venugopal and Gopakumar, 2017). In contrast, the digestive tracts of terrestrial insects intended for consumption are typically left intact during processing (Kierończyk et al., 2022). Consequently, these digestive systems may contain substantial quantities of potentially harmful microbiota, including mesophilic aerobes, Enterobacteriaceae, bacterial endospores or spore-forming bacteria, lactic acid bacteria, psychrotrophic aerobes, as well as yeasts and moulds (Martínez-Girón et al., 2017; Garofalo et al., 2019). Within these groups, species from the genera *Cronobacter*, *Bacillus*, *Clostridium*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Vibrio*, *Escherichia*, *Serratia*, *Proteus*, *Yersinia*, *Campylobacter*, *Salmonella*, *Listeria*, *Aspergillus*, *Penicillium*, *Alternaria*, *Chaetomium*, *Mucor*, *Phoma*, *Drechslera*, and *Fusarium* have been identified as potentially pathogenic or harmful.

Among these, certain species within the genera *Aspergillus* and *Penicillium*, which may be present in the insect microbiota, are known to produce mycotoxins. For example, *Aspergillus niger* and *A. flavus* produce aflatoxins, *A. ochraceus* synthesises ochratoxins, while *Penicillium citrinum* and *P. verrucosum* produce citrinin, with the latter also capable of producing ochratoxins. Mycotoxins, such as aflatoxins, are thermally stable secondary metabolites synthesised by specific fungal species. Their chemical stability at elevated temperatures renders them resistant to degradation during conventional cooking or heat-processing methods. As a result, mycotoxins persist in food products, with the potential for bioaccumulation or transfer across trophic levels within the food chain, posing significant health risks to both animals and humans. Additionally, certain bacterial species, e.g., *Bacillus* spp., *Clostridium* spp., and *Staphylococcus* spp. (including *S. aureus*), as well as fungal species such as *Debaryomyces hansenii* have been detected in ready-to-eat insect-based products (Garofalo et al., 2019). These microorganisms, present in the digestive tract at the time of slaughter, pose a potential food safety risk even when processing and preparation are carefully managed. Harmful substances absorbed, accumulated, or produced during an insect's lifecycle can remain within its tissues. Among these substances are metals, including heavy metals, whose concentrations in the insect's body can increase proportionally with their environmental availability (Moniello et al., 2019; Okrutniak and Grześ, 2021). In regions with a historical tradition of entomophagy, such as Mexico, insects are often harvested from the wild, where their diet and environmental exposure are not regulated or controlled, potentially leading to increased contamination risks (van Huis et al., 2013; Lähteenmäki-Uutela et al., 2021). However, Mexican regulations govern harvesting areas, permitting insect collection only in organic production zones or regions with minimal or no human activity, where environmental contamination is presumably lower (Lähteenmäki-Uutela et al., 2021). As a result, the bioaccumulation of harmful substances poses a significant threat to both livestock and human health when these insects are consumed directly or used as feed. Considering the nutritional potential of insects and the prospect of a lower environmental impact associated with their farming, the number of studies exploring insects as alternative food sources has markedly increased in recent decades, resulting in the need to establish parameters and standardised methods for insect health testing (van Huis, 2020; Beyers et al., 2023). In parallel, the development of such methods would support and improve regulations, as well as production systems such as GMP+ and HACCP, which are designed to ensure the quality, as well as chemical and microbiological safety of the final product.

Insects in waste bioconversion

Monitoring insect health is essential when they are utilised to bionconvert various types of human waste, particularly if their future use as food or feed becomes permissible. As scientific knowledge advances and appropriate methodologies are developed, insects may hold significant potential for sustainable applications in food and feed production systems. An example of such use is the rearing of black soldier fly larvae on agro-residues, plant-derived waste, manure or food waste (Fu et al., 2022; Beyers et al., 2023). The use of bio-waste for insect farming represents an ecologically sustainable approach, consistent with the principles of sustainable agricultural development. This concept promotes a closed-loop agricultural system by maximising resource efficiency, reducing the environmental impact compared to conventional methods, and further encouraging the use of by-products generated during insect rearing (Bulak et al., 2020; Gasco et al., 2020; Houben et al., 2020; van Huis, 2020; Fu et al., 2022; Beyers et al., 2023). Another example of the use of insects for waste disposal is research focusing on the rearing of Tenebrionidae on various types of plastics or even graphene oxide (Bulak et al., 2021; Liu et al., 2022). In such cultures, insects are fed only inorganic materials, such as various forms of plastic or a mixture of plastic and bran (Yang et al., 2018; Bulak et al., 2021; Lou et al., 2021; Peng et al., 2021). Feeding insects with a mixture of bran and plastic was found to prevent cannibalism, promote larval development and increase biodegradation (Yang et al., 2018; Lou et al., 2021; Peng et al., 2021). The biodegradation capacity of insects is related to the bacteria residing in their digestive tracts, which possess the enzymatic capacity to degrade and utilise inorganic materials (Yang et al., 2018; Lou et al., 2021; Peng et al., 2021). However, such biodegradation is not fully efficient and, depending on the primary material and differences in the composition of the microbiota population, undigested polymers are excreted with frass. While such a diet allows the larvae to develop properly to the imago stage, many studies have not verified the effect of a diet consisting of plastic on insect health, and in some cases, even on their growth rate. Meanwhile, bacteria capable of degrading inorganic polymers have been identified and isolated from the digestive tracts of insects (Brandon et al., 2021;

Park et al., 2023). This discovery may lead to the potential replacement of insects with isolated or genetically modified bacterial strains for plastic bioconversion.

Potential use of synanthropic insects as bioindicators

Insects, due to their relatively short life cycles, widespread distribution, remarkable species diversity, and integral roles in ecosystems, serve as highly suitable subjects for research into ecosystem functioning and health. Consequently, they are frequently employed as model organisms or bioindicators. Among these, social insects have been particularly recognised as potential bioindicators in ecological studies (Skaldina and Sorvari, 2017). The increasing synanthropisation of certain insect species, such as the black garden ant (*Lasius niger*), which frequently nests in close proximity to human habitats (Czechowski et al., 2012), or silverfish (*Lepisma saccharina*), which inhabits human dwellings (Joshi et al., 2020), may predispose such species to be used as bioindicators to monitor the immediate human environment. This is particularly important where interaction with xenobiotics may occur, as exemplified by the ability of ants to accumulate heavy metals in their bodies (Okrutniak and Grześ, 2021). Therefore, careful selection of research parameters and the development of standardised, straightforward methodologies are essential to enable effective monitoring of human-associated environments using insect bioindicators.

Insect health assessment in diagnostics and toxicological studies

Diagnostic methods for assessing the health status of insects have primarily been developed for bees (*Apis mellifera*), focusing on evaluating their general physiological condition or detecting viral infections (Hartfelder et al., 2013; Huang et al., 2021). In comparison, the health of other insect species has been extensively investigated in toxicological studies, although standardised methodologies are lacking. These type of studies typically aim to identify insecticides effective against pest species, including termites (*Psammotermes hypostoma*) (Allam et al., 2022), (*Odontotermes formosanus*) (Nasser et al., 2024), (*Coptorermes curvignathus*) (Kadir et al., 2023), poultry red mites (*Dermanyssus gallinae*) (Sioutas et al., 2023), red imported fire ants (*Solenopsis invicta*) (Zheng et al., 2021; Yang et al., 2023; Ma et al., 2024), pavement ants (*Tetramorium immigrans*) (Barrett et al., 2020), leaf-cutting ants (*Atta opaciceps*, *A. sexdens*, *A. sexdens rubropilosa*, *Acromyrmex lobicornis*) (Buteler et al., 2018; Rocha et al., 2018), red flour beetles (*Tribolium castaneum*) (Alif Alisha and Thangapandiyan, 2019), pink stem borers (*Sesamia inferens*) (Metwally et al., 2021), cotton leafworms (*Spodoptera littoralis*) (El-Ashmouny et al., 2022; Abdou and Zyaan, 2023), American bollworms (*Helicoverpa armigera*) (Asghar et al., 2022), and desert locusts (*Schistocerca gregaria*) (Teleb et al., 2012). Toxicological research also focuses on the effects of xenobiotics and widely used agricultural chemicals on ecologically important insects critical to ecosystem health. Such studies often target honey bees (Özkan et al., 2015; Heard et al., 2017; Tomé et al., 2020), Asian weaver ants (*Oecophylla smaragdina*) (Sheeja et al., 2020) black garden ants (*Lasius niger*) (Cuvillier-Hot et al., 2014), as well as important model species such as fruit flies (*Drosophila melanogaster*) (Ávalos et al., 2015), greater wax moths (*Galleria mellonella*) (İçen et al., 2005; Eskin et al., 2022), or silkworms (*Bombyx mori*) (Asghar et al., 2022). In addition to mortality rates and phenotypic alterations, toxicological studies frequently assess the impact of substances by analysing changes in genetic markers, enzymatic activity, and metabolite concentrations. These parameters provide valuable information regarding physiological disorders caused by xenobiotics. Developing methodologies to monitor the physiological state of reared insects could therefore benefit from the knowledge gained through toxicological research, helping to identify the most effective indicators. Biochemical assays, such as the measurements of enzymatic activity, appear particularly promising for this purpose due to their cost-effectiveness, procedural simplicity, and the availability of reagent kits that streamline performance and reduce time requirements. Additionally, these tests necessitate a relatively modest laboratory infrastructure, making them widely accessible for both research and diagnostic applications.

Biochemical assays applied on insects and significance of measured parameters in assessing insect health

Biochemical analyses in vertebrates are typically conducted using sample types, such as blood, plasma, serum, or tissue (Puppel et al., 2022; Kamaszewski et al., 2023; Łosiewicz and Szudrowicz, 2024). Blood sampling and subsequent biochemical assays are standard practices in vertebrate diagnostics.

In contrast, the relatively small size of insects presents considerable challenges in isolating organs or tissues across many species. For smaller insects, this process often requires the use of specialised equipment, such as microscopes or dissection tools, while in some cases, isolation of specific organs or tissues is highly impractical or even impossible. As a result, biochemical determinations in insects are often carried out on whole-body samples, individual dissected tissues, or eluates obtained from insect surface proteins (Table 4). These methods usually require the sacrifice of the insect, making non-lethal sampling difficult to perform. While haemolymph can be extracted without killing the insect, the volume obtained is very limited, e.g. only 0.5–1 µl can be recovered from a honey bee, as reported by Huang et al. (2021). Thus, conducting comprehensive biochemical analyses often requires the collection and sacrifice of a large number of individual insects.

Table 4. Biochemical markers that are determined in insects

Species	Used sample	Determined enzymatic activity or metabolite/ nutrient concentration	References
Asian weaver ant (*Oecophylla smaragdina*)	Worker ants	CAT, GSH, GST, MDA, SOD	Sheeja et al. (2020)
Cotton leafworm (*Spodoptera littoralis*)	Fourth instar larvae	ACP, AChE, ALP, ALT, AST, chitinase, α-esterase, β-esterase, total carbohydrates, total lipids, total proteins, digestive enzymes, PO	Assar et al. (2016)
Desert locust (*Schistocerca gregaria*)	Hemolymph and fat bodies of fifth nymphal instar and newly emerged adults	ALP, ALT, AST	Teleb et al. (2012)
Diamondback moth (*Plutella xylostella*)	Second instar larvae	AChE, CarE, GST,	Gong et al. (2013)
Greater wax moth (*Galleria mellonella*)	Larvae	AChE, ALT, AST, MDA	İçen et al. (2005)
Greater wax moth (*Galleria mellonella*)	Hemolymph of fourth instar larvae	AChE, CAT, SOD	Eskin et al. (2022)
Honey bee (*Apis mellifera*)	Hemolymph and body surface elution of worker bees	ALP, ALT, AST, total protein, protease, inhibition of proteases	Łoś and Strachecka (2018)
Honey bee (*Apis mellifera*)	Hemolymph of forager bees	Glucose, trehalose, total lipids, total protein, vitellogenin	Tlak Gajger et al. (2020)
Sunn pest (*Eurygaster integriceps*)	Hemolymph and fat bodies of imagoes	AST, ALT, ACP, ALP, EST, GST, glycogen, LDH, trehalose, total lipids, total protein, uric acid	Zibaee et al. (2011)
Pea aphid (*Acyrthosiphon pisum*)	Groups of ten imagoes	Glucose, trehalose	Wang et al. (2021)
Ponerine ant (*Harpegnathos saltator*)	Gamergates, worker ants	CAT, GPX, GSH, GSSG, GST, SOD	Schneider et al. (2011)
Red flour beetle (*Tribolium castaneum*)	Imago	ALT, AST, glucose, total lipids, total protein	Hashem et al. (2020)
Red palm weevil (*Rhynchophorus ferrugineus*)	Fat body and hemolymph of fifth instar larvae	ACP, ALP, ALT, AST	Hamadah (2019)
Texas field cricket (*Gryllus texensis*)	Hemolymph of imago	Total lipids	Adamo (2010)
Tobacco cutworm (*Spodoptera litura*) and cotton bollworm (*Helicoverpa armigera*)	100 mg of larvae's	Glucose, GST, LDH, protein oxidation, TBARS	Dixit et al. (2017)
Yellow mealworm (*Tenebrio molitor*)	Midgut of larvae and imago	α-amylase, glucosidase, proteases, inhibition of α-amylase and proteases	Dastranj et al. (2013)
Yellow mealworm (*Tenebrio molitor*)	Cell culture from imago and larvae gut	Cytochrome P450, EST, GST	Pedersen et al. (2020)

CAT – catalase, GSH – glutathione, GST – s-glutathione transferases, PO – phenoloxidase, MDA – malondialdehyde, SOD – superoxide dismutase, ACP – acid phosphatase, AChE – acetylcholine esterase, ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, CarE – carboxyesterase, LDH – lactate dehydrogenase, GPX – glutathione peroxidases, GSSG – oxidised glutathione, EST – esterases, TBARS – thiobarbituric acid reactive substances

As in vertebrates, enzymatic activity in the insect body is correlated with many factors such as age, sex, developmental stage or changes in the external environment. The relevance of specific enzymatic activity parameters or the concentration of a particular substance in the tissue under study may vary in their usefulness for evaluating the health status of insects.

Metabolite and nutrient levels, such as proteins, sugars, and lipids, provide limited diagnostic value for assessing insect health due to their susceptibility to variations influenced by nutritional status and hydration levels (Lee et al., 2008).

However, these parameters are also dependent on the physiological state of the insect and, when combined with specific enzymatic activity markers, they can provide a more precise assessment of overall insect health. Moreover, the composition and concentration of metabolites, including lipids and nutrients, in the haemolymph are influenced by the insect-associated microbiota, that may also be present in the haemolymph (Blow and Douglas, 2019). Total protein content in insect homogenates can be a useful indicator for evaluating the effects of a diet on insect development. Nevertheless, when a standardised diet is used, total protein levels alone may not provide comprehensive information about insect health. Conversely, fluctuations in total protein concentration in the haemolymph may indicate physiological conditions such as dehydration or inflammation. Additionally, protein levels in the haemolymph can be influenced by dietary composition, thereby complicating the interpretation of these results (Lee et al., 2008; Łoś and Strachecka, 2018). Similarly, haemolymph sugar concentrations are largely dependent on the insect's diet. The primary sugar present in insect haemolymph is trehalose, which functions as the main energy carrier and a glucose reservoir (Yamada et al., 2018). Apart from its metabolic role, trehalose acts as a cellular and protein bioprotectant under extreme environmental stress conditions, including thermal stress, oxidative damage, desiccation, hypoxia, and cold exposure (Elbein et al., 2003). Trehalose concentration in the haemolymph has also been shown to influence feeding behaviour, dietary preferences, and feed utilisation efficiency, highlighting its multifaceted role in insect physiology and survival (Wang et al., 2021). According to research by Jones et al. (1981), trehalose concentration also affects signalling pathways that regulate larval development in tobacco hornworm/tobacco hawk moth (*Manduca sexta*). While glucose is also present alongside trehalose in insect haemolymph, its level is significantly lower. Similar to trehalose, glucose levels are regulated in response to the insect's satiety state, with concentrations decreasing during periods of starvation and increasing following food intake (Tellis et al., 2023). Additionally, in certain insect species, haemolymph glucose levels have been shown to influence feeding behaviour (Wang et al., 2021). Insects synthesise and store glycogen in various tissues, including the fat body and muscles. Glycogen levels in insects fluctuate similarly to vertebrates, in which the liver (fat body in insects) and muscle glycogen act as sugar storage for different physiological purposes. Muscle glycogen is utilised to produce energy in the form of adenosine triphosphate through glycolysis, while fat body/liver glycogen acts as a storage and distribution centre of glucose and, uniquely in insects, trehalose, which is transported throughout the body via the haemolymph (Yamada et al., 2018).

Total body lipid content, along with total protein content, is a well-established and widely utilised parameter for evaluating the nutritional value of insects. Analysis of the fatty acid class profile in insects is used especially in experiments involving dietary and nutritional applications (Aguilar, 2021). Lipid content in the haemolymph can also be used to understand the relationship between stress response pathways and immune response signalling (Adamo, 2010). Insects mainly synthesise and store lipids in the fat body, from where they are mobilised and transported through the hemolymph to meet metabolic demands (Toprak et al., 2020). Concentration of metabolites and nutrients in both vertebrates and insects can offer valuable information on health status (Bujak et al., 2015; Sullivan et al., 2016; Łosiewicz and Szudrowicz, 2024).

Enzyme activity parameters are more specific indicators of insect health, as they are controlled by multiple signalling pathways play a critical role in maintaining homeostasis and supporting various physiological functions (Dolezal et al., 2019). For this reason, enzyme activity assays provide more precise information about the insect's health compared to metabolite and nutrient levels. Commonly determined enzymes in biochemical panels include phosphatases, transaminases, digestive enzymes, detoxifying enzymes, free radical-inactivating enzymes, and those involved in immune response.

Phosphatases are enzymes that hydrolyse phosphate monoesters in acidic or alkaline environment (Yi and Adams, 2001). In insects, acid phosphatase (ACP) is active in the intestine, Malpighian tubules, and is also found in organs and tissues undergoing cytolysis (Yi and Adams, 2001; Hamadah, 2019). In contrast, alkaline phosphatase (ALP) is a brush-border membrane enzyme, with its highest activity in tissues engaged in active membrane transport, such as intestinal epithelial cells and Malpighian tubules (Yi and Adams, 2001). ALP also plays a role in tissue cytolysis during insect development (Hamadah, 2019). The cytolytic properties of ACP and ALP make them effective markers for detecting cytolysis, particularly when induced by exposure to xenobiotics.

Transamination is the process by which amino groups are removed from amino acids and transferred to keto acid, resulting in the formation of a new amino acid from the original keto acid and the keto acid form of the initial amino acid (Litwack, 2022). This process occurs in many insect tissues, especially those containing glutamate, aspartate, and alanine (Teleb et al., 2012). It is primarily mediated by alanine aminotransferase (ALT) and aspartate aminotransferase (AST), enzymes that play a crucial role in amino acid biosynthesis, nitrogenous waste metabolism, and gluconeogenesis. These enzymes are integral to both protein anabolism and catabolism, linking carbohydrate and protein metabolism. Their activity is dynamic and subject to alteration under various physiological and pathological conditions (Nath et al., 1997). In healthy insects, the activity of ALT and AST in the haemolymph typically increases during maturation and aging. However, exposure to insecticides can lead to a reduction in ALT and AST activity, thereby disrupting essential metabolic cycles. This decline may also reflect a decrease in nutritionally significant proteins, which are required for the synthesis of amino acids necessary for tissue development, secretion processes, and energy production (Hashem et al., 2020). As in vertebrates, elevated ALT and AST activity in insects can indicate damage not only to the liver/fat body, respectively, but also other tissues (Nath et al., 1997; Puppel et al., 2022; Łosiewicz and Szudrowicz, 2024), which can be used to assess immune status (Hashem et al., 2020).

Digestive enzymes include amylases, glucosidases, proteinases, and lipases, each playing a specific role in the breakdown of carbohydrates, proteins, and lipids, respectively. Amylases are responsible for the hydrolytic breakdown of polysaccharides, primarily starch and glycogen, into maltose, maltotriose, and maltodextrins. Their activity is influenced by the insect's diet, reflecting the adaptation of digestive processes to available food sources. Products generated by amylase activity are subsequently hydrolysed by glucosidases to glucose (Da Lage, 2018). Proteinases represent the predominant group of hydrolytic enzymes in insects, playing a vital role in various physiological processes. They are mainly involved in the digestion of proteins, activation of proenzymes, release of physiologically active peptides, and activation of the complement. Additionally, proteinases contribute to inflammatory processes, underscoring their importance in both metabolic and immune functions. They are classified by their catalytic mechanism into serine proteinases, cysteine proteinases, aspartate proteinases and metalloproteinases, of which serine and cysteine proteinases are the main classes of proteinases in phytophagous insects (Macedo and Freire, 2011). Lipases play key roles in insects, facilitating lipid acquisition, storage, and distribution, while also being involved in many physiological processes (Santana et al., 2017). The activity of digestive enzymes, including lipases, may be influenced by dietary factors, including inhibition potentially caused by xenobiotics produced by plants in an area under study (Macedo and Freire, 2011; Dastranj et al., 2013). Moreover, the activity of digestive enzymes can be modulated by the insect's microbiota and developmental stage (Dastranj et al., 2013; Gandotra et al., 2018).

Lactate dehydrogenase (LDH) catalyses the reversible conversion of pyruvate to lactate along with the interconversion of reduced nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide. LDH facilitates this process in the absence or insufficient oxygen levels. Elevated LDH levels are associated with tissue breakdown (Zibaee et al., 2011), making it a reliable indicator of tissue and organ damage in in vitro cultures and animal studies. Research on insects has shown that LDH activity in muscles is generally low in most species, and that α-glycerophosphate dehydrogenase (GPDH) shows a higher activity, and thus plays a greater role in the metabolic pathways of muscle function (Zebe and McShan, 1957; Kitto and Briggs, 1962). Interestingly, species with higher LDH activity in muscles tend to have lower GDH I activity (Zebe and McShan, 1957). These species include insects that rely on strong legs rather than wings for locomotion (Kitto and Briggs, 1962). LDH activity measurements has been utilised as an indicator of exposure to chemical stress in toxicological studies (Zibaee et al., 2011; Kissoum and Soltani, 2016).

Chitinases are enzymes that hydrolyse chitin, a major structural component of arthropod exoskeletons and fungal cell walls. These enzymes are found in various organisms, including bacteria, protists, fungi, plants and animals, such as arthropods and mammals. Chitinases perform various functions, including involvement in digestion, arthropod moulting, immunity and pathogenicity. The majority of insect chitinases are endochitinases, which are essential for normal insect growth and development. Their concentration increases significantly before moulting (Arakane and Muthukrishnan, 2010), when the insect sheds its old chitinous exoskeleton and replaces it with a new, larger one.

Detoxification enzymes such as cytochrome P450 enzymes, esterases (EST), including carboxyesterase (CarE), acetylcholine esterase (AChE), α-esterase, and β-esterase, as well as S-glutathione transferases (GST) represent another key group studied in insect health research. These enzymes form the three primary systems responsible for the detoxification of xenobiotics (Saha et al., 2012).

As in vertebrates, cytochrome P450 enzymes in insects constitute a diverse group of oxidases involved in the metabolism of many substances. These enzymes can be found in virtually every insect tissue and they perform various important functions, ranging from participation in the synthesis and degradation of hormones to the metabolism of xenobiotics. Assessing cytochrome P450 enzymes typically involves determining the expression levels of genes encoding them, as it provides information about the regulation of their synthesis (Piechowicz et al., 2021; Xiong et al., 2022; Koto et al., 2023); however, enzymatic measurements are also frequently employed in research (Xiong et al., 2019; Pedersen et al., 2020).

Esterases, similarly to cytochrome P450 enzymes, are present in many insect tissues and play a role in the metabolism of xenobiotics in the insect body (Sezer Tuncsoy et al., 2019). These enzymes hydrolyse metabolised substances and may also participate in the digestion of ingested food (Bhatt et al., 2020). The specific role of individual EST in metabolism is not fully elucidated and requires further research into their functions in substance metabolism (Jackson et al., 2013). However, certain EST can be distinguished based on their substrate specificity and physiological roles. For instance, AChE is responsible for the hydrolysis of acetylcholine into choline and acetate, playing a critical role in the regulation of the cholinergic system (Sezer Tuncsoy et al., 2019). Additionally, juvenile hormone EST, a member of the carboxylesterase family, is involved in the metabolism of juvenile hormone, a key regulator of insect development and reproduction (Kamita and Hammock, 2010).

S-glutathione transferases are a group of detoxifying enzymes present in both insects and vertebrates. These enzymes catalyse the conjugation of reduced glutathione (GSH) to electrophilic substrates in biotransformation processes. The resulting conjugates are subsequently excreted from the cell via the glutathione S-conjugate secretion pump and transformed into excretable mercapturic acids (Simon, 1996). In addition, s-glutathione transferases are also involved in the oxidative stress response by catalysing hydroperoxides (ROOH) reduction reactions (without hydrogen peroxide (H_2O_2) as a substrate) (Felton and Summers, 1995).

Enzymes comprising the three main systems involved in the detoxification of xenobiotics play a crucial role in insect resistance to insecticides. For this reason, their impact on the effectiveness of artificial insecticides (Gong et al., 2013; Xiong et al., 2019, 2022; Jaffar et al., 2022), as well as on the efficiency of insecticides naturally secreted by plants, is often evaluated (Saha et al., 2012). Additionally, tests measuring the activity of detoxification enzymes are used to assess the impact of xenobiotics, such as nanoparticles, with GST activity commonly studied due to its involvement in the oxidative stress response (Sezer Tuncsoy et al., 2019; Sheeja et al., 2020; Muhammad et al., 2022). Moreover, the insect's microbiota and its associated detoxifying enzymes may also significantly contribute to its pesticide resistance (Jaffar et al., 2022).

The generation of oxygen free radicals in the mitochondria is essential for cellular function. However, to prevent their uncontrolled action, which can lead to the oxidation of fats, proteins, and ultimately cell death, organisms have developed defence mechanisms. Antioxidant defences in insects consist of catalase (CAT), glutathione peroxidases (GPX), glutathione reductase (GR), superoxide dismutase (SOD) and S-glutathione transferases (Felton and Summers, 1995). Additionally, GST is also involved in detoxification (Simon, 1996).

Superoxide dismutase catalyses the conversion of oxygen radicals into hydrogen peroxide, which is subsequently converted to water by catalases or glutathione peroxidases (Felton and Summers, 1995; Paes et al., 2001). Glutathione peroxidases not only catalyse the reduction of hydrogen peroxide, but also reduce lipid hydroperoxides (Felton and Summers, 1995; Dias et al., 2016). Similarly, s-glutathione transferases catalyse the reduction reaction of organic peroxides. In both cases, glutathione (GSH) is utilised and converted into oxidised glutathione (GSSG). Glutathione levels are then restored through the conversion of GSSG by glutathione reductase (Felton and Summers, 1995). Simultaneously, a lack of GPX enzyme activity has been observed in certain insect species, accompanied by abnormal distribution of CAT and GR in the cells. These findings suggest an evolutionary adaptation or compensatory mechanism to mitigate the lack of GPX (Ahmad et al., 1988). In addition, these enzymes are also involved in neutralising the action of free radicals that have been absorbed from the environment (Paes et al., 2001). CAT, GPX, GR, GST and SOD are also frequently used as indicators of oxidative stress induced by xenobiotics in both insects (Sezer Tuncsoy et al., 2019; Muhammad et al., 2022) and vertebrates (Anreddy, 2018; Kamaszewski et al., 2023).

Additional markers of oxidative stress include the determination of antioxidants such as GSH, a well-known tripeptide that reacts directly or acts as a co-substrate in oxidative stress defence reactions (Paes et al., 2001), as well as uric acid, GSSG, or the measurements of oxidised protein and lipid levels. When antioxidant enzymes are ineffective, reactive oxygen species (ROS) accumulate in the cell, initiating reactions with proteins, nucleic acids, and lipids (Orčić et al., 2017). Free radicals, in particular, react with polyunsaturated fatty acids, such as cell membrane phospholipids, leading to the formation of lipid peroxides. The concentration of peroxidised lipids is commonly measured using one of two methods: the quantification of malondialdehyde (MDA) or the measurement of thiobarbituric acid reactive substances (TBARS). MDA is a reactive compound formed during lipid peroxidation and is widely considered a reliable marker of oxidative stress (İçen et al., 2005; Orčić et al., 2017). Similarly, TBARS are products of lipid peroxidation, formed through the reaction of thiobarbituric acid with aldehydes such as MDA and hydroxynonenals, which represent the breakdown of peroxidation products (Ahmad et al., 1995).

In insect organisms, uric acid is used as a nitrogenous waste, nitrogen storage, pigment, antioxidant (Tasaki et al., 2017), and potentially as a signalling molecule (Weihrauch and O'Donnell, 2021). Uric acid synthesis is one of the most common mechanisms to avoid the toxic effects of ammonia derived from the catabolism of proteins and nucleic acids in insects; however, some species not subjected to water stress can excrete ammonia directly. On the other hand, certain dipterans can further metabolise uric acid to allantoin or urea (Weihrauch and O'Donnell, 2021). The two main sources of uric acid synthesis in insects are the uricotelic pathway, where proteins provide the nitrogen source, and purine degradation metabolism. Moreover, uric acid inhibits the generation of oxygen free radicals in insects by chelating metals and scavenging free radicals once they are generated. In addition, it has the ability to form stable coordination complexes with iron ions which reduces free radical production and limits ascorbate oxidation (Weihrauch and O'Donnell, 2021). In a study conducted by Tasaki et al. (2017), termites (*Reticulitermes speratus*) showed higher antioxidant activity than other species, which could be related to their longevity compared to other insects. Moreover, the longer they were isolated from the colony, and thus exposed to UV light, the more they turned white, which was associated with higher uric acid levels and significantly lower free radical concentrations, as well as enhanced antioxidant activity. In vertebrates, uric acid is also a nitrogenous waste product, but it is also known for its beneficial antioxidant and neuroprotective properties (Álvarez-Lario and Macarrón-Vicente, 2010).

Vitellogenin and phenoloxidase (PO) are employed as markers of immune response in insects. Vitellogenin is an important animal protein that is not only a major precursor of yolk proteins in insects and other oviparous species (Tufail et al., 2014), but also has immune functions and antioxidant properties by neutralising free radicals (Salmela and Sundström, 2017). Its antioxidant action is known to contribute to the longer lifespan of queens and long-lived workers in honey bees (Salmela and Sundström, 2017; Wu et al., 2021). Additionally, vitellogenin possesses anti-inflammatory properties by binding to damaged cells through lipids, especially phosphatidylserine. Similar to fish vitellogenin, insect vitellogenin binds to bacteria by interacting with their lipoproteins. In addition, it interacts directly with insect immune cells – haemocytes – likely providing them with zinc ions necessary for their proper functioning (Salmela and Sundström, 2017). Mammals, including humans, have homologous proteins involved in fat metabolism and immunity, although they are not involved in reproduction (Salmela and Sundström, 2017). Phenoloxidase is an enzyme with a crucial function in insect humoral defence. It is well documented as a copper-containing enzyme and is also found in microorganisms, plants and other animals (Marieshwari et al., 2023). Phenoloxidase is involved in melanin formation (Assar et al., 2016), sclerotisation, opsonisation and wound healing. In addition, in insects, it plays key roles during moulting, non-self/

self-recognition, phagocytosis and nodule formation (Marieshwari et al., 2023). In insects, PO is typically present in its zymogen form as profenoloxidase (proPO), which is proteolysed by serine protease to active PO. Interestingly, serine protease is also found in zymogen form and requires activation through a protease influx induced by infection. This mode of proPO activation mechanism helps prevent tissue damage caused by PO, which in its active form can react even with oxygen free radicals produced during normal metabolism. Prolonged activation of proPO leads to oxidative damage (Marieshwari et al., 2023).

Summary

The health status of insects used in the production of insect meal for feed is a frequently overlooked aspect in both husbandry practices and research. Considering the potential impact of insect health on the quality of feed and, consequently, on livestock welfare, we have evaluated biochemical methods commonly applied in insect research to determine their utility for assessing insect health. Analyses conducted on individual insect tissues provide the most detailed and specific information about the physiological condition of insects. However, biochemical assays performed on whole insects or even pooled groups of individuals can also yield reliable results, offering faster processing and applicability to species of any size. For both approaches, it is crucial to randomly select a representative group of insects from the cultured population, as sampling typically requires sacrificing the insects. An exception is the collection of small volumes of haemolymph (0.5–1 µl), though this amount may be insufficient for comprehensive biochemical analyses. Measurements of metabolites and nutrients, while less directly informative about overall insect health, serve as valuable supplements to assays determining enzymatic activity and levels of substances involved in antioxidant or immune defence. Collectively, these methods would provide a robust framework for monitoring the health of insects used in feed production and experiments.

Conclusions

In conclusion, we propose that biochemical panels for diagnosing insect health status should include assays for the activity of enzymes, such as phosphatases, transaminases, antioxidant enzymes, and detoxifying enzymes, alongside markers associated with immune responses. These tests are particularly critical in cases where xenobiotic intoxication or infection by insect pathogens is suspected. Additionally, the measurement of total protein content should be included to standardise samples, with the possibility of adding other metabolites based on specific research or diagnostic requirements.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

Abd-El Wahed S.M.N., Ahmad A.F., 2019. Variations in chemical composition value of adults and nymphs desert locust, *Schistocerca gregaria* Forskal (Orthoptera: Acrididae). J. Plant Prot. Pathol. 10, 677–681, https://doi.org/10.21608/jppp.2019.79461

Abdou M., Zyaan O., 2023. The proficiency of silver nanoparticles in controlling cotton leafworm, *Spodoptera littoralis* (Boisd.), under the laboratory conditions. Egypt. J. Zool. 80, 1–17, https://doi.org/10.21608/ejz.2022.174453.1090

Adámková A., Kouřimská L., Borkovcová M., Kulma M., Mlček J., 2016. Nutritional valuse of edible coleoptera (*Tenebrio molitor*, *Zophobas morio* and *Alphitobius diaperinus*) reared reared in the Czech Republic. Potravin. Slovak J. Food Sci. 10, 663−671, https://doi.org/10.5219/609

Adamo S.A., 2010. Why should an immune response activate the stress response? Insights from the insects (the cricket *Gryllus texensis*). Brain. Behav. Immun. 24, 194–200, https://doi.org/10.1016/j.bbi.2009.08.003

Adli D.N., 2021. Use of insects in poultry feed as replacement soya bean meal and fish meal in development countries: a systematic review. Livest. Res. Rural Dev. 33, 10

Aguilar J.G. dos S., 2021. An overview of lipids from insects. Biocatal. Agric. Biotechnol. 33, 101967, https://doi.org/10.1016/j.bcab.2021.101967

Ahmad S., Pritsos C.A., Bowen S.M., Heisler C.R., Blomquist G.J., Pardini R.S., 1988. Antioxidant enzymes of larvae of the cabbage looper moth, *Trzchoplusza Ni*: Subcellular distribution and activities of superoxide dismutase, catalase and glutathione reductase. Free Radic. Res. Commun. 4, 403–408, https://doi.org/10.3109/10715768809066908

Ahmad S., Zaman K., MacGill R.S., Batcabe J.P., Pardini R.S., 1995. Dichlone-induced oxidative stress in a model insect species, *Spodoptera eridania*. Arch. Environ. Contam. Toxicol. 29, 442–448, https://doi.org/10.1007/BF00208372

Al-Homidan I.H., Basha N.E., Abou-Emera O.K., Ebeid T.A., Al-Waily S.M., Alamer S.S., Fathi M.M., 2024. Effects of Desert locust dietary supplementation (*Schistocerca gregaria*) on growth performance, carcass quality and blood biochemistry of broiler chickens. Egypt. Poult. Sci. J. 44, 231–242, https://doi.org/10.21608/epsj.2024.363435

Alif Alisha A.S., Thangapandiyan S., 2019. Comparative bioassay of silver nanoparticles and malathion on infestation of red flour beetle, *Tribolium castaneum*. J. Basic Appl. Zool. 80, 55, https://doi.org/10.1186/s41936-019-0124-0

Allam R.O.H., Badawy A.M.M., Ali M.A., 2022. Green synthesized silver nanoparticles for controlling subterranean termites, *Psammotermes hypostoma* (Desn.). SVU-International J. Agric. Sci. 4, 135–143, https://doi.org/10.21608/svuijas.2022.116350.1170

Álvarez-Lario B., Macarrón-Vicente J., 2010. Uric acid and evolution. Rheumatology 49, 2010–2015, https://doi.org/10.1093/rheumatology/keq204

Anankware J.P., Roberts B.J., Cheseto X., Osuga I., Savolainen V., Collins C.M., 2021. The nutritional profiles of five important edible insect species from West Africa-An analytical and literature synthesis. Front. Nutr. 8, 792941, https://doi.org/10.3389/fnut.2021.792941

Anreddy R.N.R., 2018. Copper oxide nanoparticles induces oxidative stress and liver toxicity in rats following oral exposure. Toxicol. Rep. 5, 903–904, https://doi.org/10.1016/j.toxrep.2018.08.022

Arakane Y., Muthukrishnan S., 2010. Insect chitinase and chitinase-like proteins. Cell. Mol. Life Sci. 67, 201–216, https://doi.org/10.1007/s00018-009-0161-9

Asghar M.S., Sarwar Z.M., Almadiy A.A., Shami A., El Hadi Mohamed R.A., Ahmed N., Waghulade M.S., Alam P., Abd Al Galil F.M., 2022. Toxicological effects of silver and zinc oxide nanoparticles on the biological and life table parameters of *Helicoverpa armigera* (Noctuidae: Lepidoptera). Agriculture 12, 1744, https://doi.org/10.3390/agriculture12101744

Assar A.A., Abo El-Mahasen M.M., Dahi H.F., Amin H.S., 2016. Biochemical effects of some insect growth regulators and bioinsecticides against cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera Noctuidae). J. Biosci. Appl. Res. 2, 587−594, https://doi.org/10.21608/jbaar.2016.108937

Ávalos A., Haza A.I., Drosopoulou E., Mavragani-Tsipidou P., Morales P., 2015. *In vivo* genotoxicity assesment of silver nanoparticles of different sizes by the Somatic Mutation and Recombination Test (SMART) on *Drosophila*. Food Chem. Toxicol. 85, 114–119, https://doi.org/10.1016/j.fct.2015.06.024

Barrett M., Caponera V., McNair C., O'Donnell S., Marenda D.R., 2020. Potential for use of erythritol as a socially transferrable ingested insecticide for ants (Hymenoptera: Formicidae). J. Econ. Entomol. 113, 1382–1388, https://doi.org/10.1093/jee/toaa019

Bartucz T., Csókás E., Nagy B., Gyurcsák M.P., Bokor Z., Bernáth G., Molnár J., Urbányi B., Csorbai B., 2023. Black soldier fly (*Hermetia illucens*) meal as direct replacement of complex fish feed for rainbow trout (*Oncorhynchus mykiss*) and African catfish (*Clarias gariepinus*). Life 13, 1978, https://doi.org/10.3390/life13101978

Bednářová M., Borkovcová M., Komprda T., 2014. Purine derivate content and amino acid profile in larval stages of three edible insects. J. Sci. Food Agric. 94, 71–76, https://doi.org/10.1002/jsfa.6198

Bessa L.W., Pieterse E., Sigge G., Hoffman L.C., 2020. Insects as human food; from farm to fork. J. Sci. Food Agric. 100, 5017–5022, https://doi.org/10.1002/jsfa.8860

Beyers M., Coudron C., Ravi R., Meers E., Bruun S., 2023. Black soldier fly larvae as an alternative feed source and agro-waste disposal route - A life cycle perspective. Resour. Conserv. Recycl. 192, 106917, https://doi.org/10.1016/j.resconrec.2023.106917

Bhatt P., Bhatt K., Huang Y., Lin Z., Chen S., 2020. Esterase is a powerful tool for the biodegradation of pyrethroid insecticides. Chemosphere 244, 125507, https://doi.org/10.1016/j.chemosphere.2019.125507

Biasato I., De Marco M., Rotolo L. et al., 2016. Effects of dietary *Tenebrio molitor* meal inclusion in free-range chickens. J. Anim. Physiol. Anim. Nutr. (Berl). 100, 1104–1112, https://doi.org/10.1111/jpn.12487

Blow F., Douglas A.E., 2019. The hemolymph microbiome of insects. J. Insect Physiol. 115, 33–39, https://doi.org/10.1016/j.jinsphys.2019.04.002

Brandon A.M., Garcia A.M., Khlystov N.A., Wu W.-M., Criddle C.S., 2021. Enhanced Bioavailability and microbial biodegradation of polystyrene in an enrichment derived from the gut microbiome of *Tenebrio molitor* (mealworm larvae). Environ. Sci. Technol. 55, 2027–2036, https://doi.org/10.1021/acs.est.0c04952

Bujak R., Struck-Lewicka W., Markuszewski M.J., Kaliszan R., 2015. Metabolomics for laboratory diagnostics. J. Pharm. Biomed. Anal. 113, 108–120, https://doi.org/10.1016/j.jpba.2014.12.017

Bulak P., Proc K., Pawłowska M., Kasprzycka A., Berus W., Bieganowski A., 2020. Biogas generation from insects breeding post production wastes. J. Clean. Prod. 244, 118777, https://doi.org/10.1016/j.jclepro.2019.118777

Bulak P., Proc K., Pytlak A., Puszka A., Gawdzik B., Bieganowski A., 2021. Biodegradation of different types of plastics by *Tenebrio molitor* insect. Polymers (Basel). 13, 3508, https://doi.org/10.3390/polym13203508

Buteler M., Lopez Garcia G., Stadler T., 2018. Potential of nanostructured alumina for leaf-cutting ants *Acromyrmex lobicornis* (Hymenoptera: Formicidae) management. Austral Entomol. 57, 292–296, https://doi.org/10.1111/aen.12277

Cerreta A.J., Smith D.C., Ange-Van Heugten K., Minter L.J., 2022. Comparative nutrient analysis of four species of cockroaches used as food for insectivores by life stage, species, and sex. Zoo Biol. 41, 26–33, https://doi.org/10.1002/zoo.21647

Cho J.H., Kim I.H., 2011. Fish meal - nutritive value. J. Anim. Physiol. Anim. Nutr. (Berl). 95, 685–692, https://doi.org/10.1111/j.1439-0396.2010.01109.x

Costa S., Pedro S., Lourenço H., Batista I., Teixeira B., Bandarra N.M., Murta D., Nunes R., Pires C., 2020. Evaluation of *Tenebrio molitor* larvae as an alternative food source. NFS J. 21, 57–64, https://doi.org/10.1016/j.nfs.2020.10.001

Cuvillier-Hot V., Salin K., Devers S., Tasiemski A., Schaffner P., Boulay R., Billiard S., Lenoir A., 2014. Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant *Lasius niger*. Environ. Res. 131, 104–110, https://doi.org/10.1016/j.envres.2014.03.016

Czechowski W., Radchenko A., Czechowska W., Vepsa-la-inen K., 2012. The ants of Poland: with reference to the myrmecofauna of Europe. Natura Optima dux Foundation. Warsaw (Poland)

Da Lage J.-L., 2018. The amylases of insects. Int. J. Insect Sci. 10, 117954331880478, https://doi. org/10.1177/1179543318804783

Dastranj M., Bandani A.R., Mehrabadi M., 2013. Age-specific digestion of *Tenebrio molitor* (Coleoptera: Tenebrionidae) and inhibition of proteolytic and amylolytic activity by plant proteinaceous seed extracts. J. Asia. Pac. Entomol. 16, 309–315, https://doi.org/10.1016/j.aspen.2013.04.009

Dias F.A., Gandara A.C.P., Perdomo H.D. et al., 2016. Identification of a selenium-dependent glutathione peroxidase in the blood-sucking insect *Rhodnius prolixus*. Insect Biochem. Mol. Biol. 69, 105–114, https://doi.org/10.1016/j.ibmb.2015.08.007

Dixit G., Praveen A., Tripathi T., Yadav V.K., Verma P.C., 2017. Herbivore-responsive cotton phenolics and their impact on insect performance and biochemistry. J. Asia. Pac. Entomol. 20, 341–351, https://doi.org/10.1016/j.aspen.2017.02.002

Dolezal T., Krejcova G., Bajgar A., Nedbalova P., Strasser P., 2019. Molecular regulations of metabolism during immune response in insects. Insect Biochem. Mol. Biol. 109, 31–42, https://doi.org/10.1016/j.ibmb.2019.04.005

EFSA Panel on Nutrition, Novel Foods and Allergens (NDA), Turck D., Castenmiller J. et al., 2021a. Safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA J. 19, e06343, https://doi.org/10.2903/j.efsa.2021.6343

EFSA Panel on Nutrition, Novel Foods and Allergens (NDA), Turck D., Castenmiller J. et al., 2021b. Safety of frozen and dried formulations from migratory locust (*Locusta migratoria*) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA J. 19, e06667, https://doi.org/10.2903/j.efsa.2021.6667

El-Ashmouny R.S., Rady M.H., Merdan B.A., El-Sheikh T.A.A., Hassan R.E., El Gohary E.G.E., 2022. Larvicidal and pathological effects of green synthesized silver nanoparticles from *Artemisia herba-alba* against *Spodoptera littoralis* through feeding and contact application. Egypt. J. Basic Appl. Sci. 9, 239–253, https://doi.org/10.1080/2314808X.2022.2063012

Elbein A.D., Pan Y.T., Pastuszak I., Carroll D., 2003. New insights on trehalose: a multifunctional molecule. Glycobiology 13, 17R–27R, https://doi.org/10.1093/glycob/cwg047

Eskin A., Ekremoglu M., Altinkaynak C., Özdemir N., 2022. Effects of organic-inorganic hybrid nanoflowers' framework on hemocytes and enzymatic responses of the model organism, *Galleria mellonella* (Lepidoptera: Pyralidae). Int. J. Trop. Insect Sci. 42, 333–344, https://doi.org/10.1007/s42690-021-00551-2

European Commission D.-G. for H. and F.S.S., 2023a. Commission Implementing Regulation (EU) 2023/5 of 3 January 2023 authorising the placing on the market of *Acheta domesticus* (house cricket) partially defatted powder as a novel food and amending Implementing Regulation (EU) 2017/2470. Off. J. Eur. Union L 2/9

European Commission D.-G. for H. and F.S.S., 2023b. Commission Implementing Regulation (EU) 2023/58 of 5 January 2023 authorising the placing on the market of the frozen, paste, dried and powder forms of *Alphitobius diaperinus* larvae (lesser mealworm) as a novel food and amending Implementing Regulation. Off. J. Eur. Union L 5/10

European Commission D.-G. for H. and F.S.S., 2021a. Commission Implementing Regulation (EU) 2021/405 of 24 March 2021 laying down the lists of third countries or regions thereof authorised for the entry into the Union of certain animals and goods intended for human consumption in accordance with Regulation. Off. J. Eur. Union L 114/118

European Commission D.-G. for H. and F.S.S., 2021b. Commission Regulation (EU) 2021/1925 of 5 November 2021 amending certain Annexes to Regulation (EU) No 142/2011 as regards the requirements for placing on the market of certain insect products and the adaptation of a containment method (Text with EEA relevance). Off. J. Eur. Union L 393/4

European Commission D.-G. for H. and F.S.S., 2017a. Regulation (EU) No 2017/893 of 24 May 2017 Amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as Regards the Provisions on processed animal protein (Text with EEA relevance). Off. J. Eur. Union L 138/92

European Commission D.-G. for H. and F.S.S., 2017b. Regulation (EU) No 2017/1017 of 15 June 2017 Amending Regulation (EU) No 68/2013 on the Catalogue of Feed Materials. Off. J. Eur. Union L 159/48

European Parliament C. of the E.U.D.-G. for H. and F.S., 2015. Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (Text with EEA relevance). Off. J. Eur. Union L 327/1

European Parliament C. of the E.U.D.-G. for H. and F.S., 2005. Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. Off. J. Eur. Union L 35/1

European Parliament C. of the E.U.D.-G. for H. and F.S., 2004. Regulation (EC) No 852/2004 of the european parliament and of the council of 29 April 2004 on the hygiene of foodstuffs. Off. J. Eur. Communities L 139/1

European Parliament C. of the E.U.D.-G. for H. and F.S., 2002a. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on Undesirable Substances in Animal Feed. Off. J. L 140, 30/05/2002, 0010-0022

European Parliament C. of the E.U.D.-G. for H. and F.S., 2002b. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety Off. J. L 031, 01/02/2002, 0001-0024

Felton G.W., Summers C.B., 1995. Antioxidant systems in insects. Arch. Insect Biochem. Physiol. 29, 187–197, https://doi.org/10.1002/arch.940290208

Finke M.D., 2013. Complete Nutrient content of four species of feeder insects. Zoo Biol. 32, 27–36, https://doi.org/10.1002/zoo.21012

Freel T.A., McComb A., Koutsos E.A., 2021. Digestibility and safety of dry black soldier fly larvae meal and black soldier fly larvae oil in dogs. J. Anim. Sci. 99, skab047, https://doi.org/10.1093/jas/skab047

Fu S.-F., Wang D.-H., Xie Z., Zou H., Zheng Y., 2022. Producing insect protein from food waste digestate via black soldier fly larvae cultivation: A promising choice for digestate disposal. Sci. Total Environ. 830, 154654, https://doi.org/10.1016/j.scitotenv.2022.154654

Gandotra S., Bhuyan P.M., Gogoi D.K., Kumar A., Subramanian S., 2018. Screening of nutritionally important gut bacteria from the lepidopteran insects through qualitative enzyme assays. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 88, 329–337, https://doi.org/10.1007/s40011-016-0762-7

Garofalo C., Milanović V., Cardinali F., Aquilanti L., Clementi F., Osimani A., 2019. Current knowledge on the microbiota of edible insects intended for human consumption: A state-of-the-art review. Food Res. Int. 125, 108527, https://doi.org/10.1016/j.foodres.2019.108527

Gasco L., Biancarosa I., Liland N.S., 2020. From waste to feed: A review of recent knowledge on insects as producers of protein and fat for animal feeds. Curr. Opin. Green Sustain. Chem. 23, 67–79, https://doi.org/10.1016/j.cogsc.2020.03.003

Gong Y.-J., Wang Z.-H., Shi B.-C., Kang Z.-J., Zhu L., Jin G.-H., Weig S.-J., 2013. Correlation between pesticide resistance and enzyme activity in the diamondback moth, *Plutella xylostella*. J. Insect Sci. 13, 135, https://doi.org/10.1673/031.013.13501

Haber M., Mishyna M., Martinez J.J.I., Benjamin O., 2019. The influence of grasshopper (*Schistocerca gregaria*) powder enrichment on bread nutritional and sensorial properties. LWT 115, 108395, https://doi.org/10.1016/j.lwt.2019.108395

Hamadah K.S., 2019. Disturbance of phosphatase and transaminase activities in grubs of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) by certain insecticidal compounds. J. Basic Appl. Zool. 80, 52, https://doi.org/10.1186/s41936-019-0123-1

Hartfelder K., Bitondi M.M.G., Brent C.S., Guidugli-Lazzarini K.R., Simões Z.L.P., Stabentheiner A., Tanaka É.D., Wang Y., 2013. Standard methods for physiology and biochemistry research in *Apis mellifera*. J. Apic. Res. 52, 1–48, https://doi.org/10.3896/IBRA.1.52.1.06

Hartmann C., Shi J., Giusto A., Siegrist M., 2015. The psychology of eating insects: A cross-cultural comparison between Germany and China. Food Qual. Prefer. 44, 148–156, https://doi.org/10.1016/j.foodqual.2015.04.013

Hashem A.S., Ramadan M.M., Abdel-Hady A.A.A., Sut S., Maggi F., Dall'Acqua S., 2020. Pimpinella anisum essential oil nanoemulsion toxicity against *Tribolium castaneum*? Shedding light on Its interactions with aspartate aminotransferase and alanine aminotransferase by molecular docking. Molecules 25, 4841, https://doi.org/10.3390/molecules25204841

Heard M.S., Baas J., Dorne J.-L., Lahive E., Robinson A.G., Rortais A., Spurgeon D.J., Svendsen C., Hesketh H., 2017. Comparative toxicity of pesticides and environmental contaminants in bees: Are honey bees a useful proxy for wild bee species? Sci. Total Environ. 578, 357–365, https://doi.org/10.1016/j.scitotenv.2016.10.180

Hong J., Han T., Kim Y.Y., 2020. Mealworm (*Tenebrio molitor larvae*) as an alternative protein source for monogastric animal: A review. Animals 10, 2068, https://doi.org/10.3390/ani10112068

Houben D., Daoulas G., Faucon M.-P., Dulaurent A.-M., 2020. Potential use of mealworm frass as a fertilizer: Impact on crop growth and soil properties. Sci. Rep. 10, 4659, https://doi.org/10.1038/s41598-020-61765-x

Huang C., Feng W., Xiong J., Wang T., Wang W., Wang C., Yang F., 2019. Impact of drying method on the nutritional value of the edible insect protein from black soldier fly (*Hermetia illucens* L.) larvae: amino acid composition, nutritional value evaluation, in vitro digestibility, and thermal properties. Eur. Food Res. Technol. 245, 11–21, https://doi.org/10.1007/s00217-018-3136-y

Huang S., Li J., Zhang Y. et al., 2021. A novel method for the detection and diagnosis of virus infections in honey bees. J. Virol. Methods 293, 114163, https://doi.org/10.1016/j.jviromet.2021.114163

Iaconisi V., Marono S., Parisi G., Gasco L., Genovese L., Maricchiolo G., Bovera F., Piccolo G., 2017. Dietary inclusion of *Tenebrio molitor* larvae meal: Effects on growth performance and final quality treats of blackspot sea bream (*Pagellus bogaraveo*). Aquaculture 476, 49–58, https://doi.org/10.1016/j.aquaculture.2017.04.007

İçen E., Armutçu F., Büyükgüzel K., Gürel A., 2005. Biochemical stress indicators of greater wax moth exposure to organophosphorus insecticides. J. Econ. Entomol. 98, 358–366, https://doi.org/10.1093/jee/98.2.358

Jackson C.J., Liu J.-W., Carr P.D. et al., 2013. Structure and function of an insect α-carboxylesterase (*α Esterase 7*) associated with insecticide resistance. Proc. Natl. Acad. Sci. 110, 10177–10182, https://doi.org/10.1073/pnas.1304097110

Jaffar S., Ahmad S., Lu Y., 2022. Contribution of insect gut microbiota and their associated enzymes in insect physiology and biodegradation of pesticides. Front. Microbiol. 13, 979383, https://doi.org/10.3389/fmicb.2022.979383

Jajić I., Krstović S., Petrović M., Urošević M., Glamočić D., Samardžić M., Popović A., Guljaš D., 2022. Changes in the chemical composition of the yellow mealworm (*Tenebrio molitor* L.) reared on different feedstuffs. J. Anim. Feed Sci. 31, 191–200, https://doi.org/10.22358/jafs/147848/2022

Janssen R.H., Vincken J.-P., van den Broek L.A.M., Fogliano V., Lakemond C.M.M., 2017. Nitrogen-to-Protein Conversion Factors for Three Edible Insects: *Tenebrio molitor*, A*lphitobius diaperinus*, and *Hermetia illucens*. J. Agric. Food Chem. 65, 2275–2278, https://doi.org/10.1021/acs.jafc.7b00471

Jeong S.-M., Khosravi S., Mauliasari I.R., Lee S.-M., 2020. Dietary inclusion of mealworm (*Tenebrio molitor*) meal as an alternative protein source in practical diets for rainbow trout (*Oncorhynchus mykiss*) fry. Fish. Aquat. Sci. 23, 12, https://doi.org/10.1186/s41240-020-00158-7

Jones D., Jones G., Bhaskaran G., 1981. Dietary sugars, hemolymph trehalose levels, and supernumerary molting of manduca sexta larvae. Physiol. Zool. 54, 260–266, https://doi.org/10.1086/physzool.54.2.30155828

Joshi M.J., Varadharasu P.R., Solanki B.C., Birari V.V., 2020. Silverfish (*Lepisma saccharina*): An overview and their management. Agric. Foods e-Newsletter 2, 490–493

Józefiak A., Kierończyk B., Rawski M., Mazurkiewicz J., Benzertiha A., Gobbi P., Nogales-Merida S., Świątkiewicz S., Józefiak D., 2018. Full-fat insect meals as feed additive - the effect on broiler chicken growth performance and gastrointestinal tract microbiota. J. Anim. Feed Sci. 27, 131–139, https://doi.org/10.22358/jafs/91967/2018

Kadir R., Li W.Y., Mat Arip M.N., Lee H.L., 2023. Incorporation of permethrin into chitosan polymeric nanoparticles using nanoprecipitation method for rubberwood preservation against termite attack. Wood Mater. Sci. Eng. 18, 1562–1572, https://doi.org/10.1080/17480272.2022.2164741

Kamaszewski M., Kawalski K., Wiechetek W. et al., 2023. The Effect of silver nanoparticles on the digestive system, gonad morphology, and physiology of butterfly splitfin (*Ameca splendens*). Int. J. Mol. Sci. 24, 14598, https://doi.org/10.3390/ijms241914598

Kamita S.G., Hammock B.D., 2010. Juvenile hormone esterase: biochemistry and structure. J. Pestic. Sci. 35, 265–274, https://doi.org/10.1584/jpestics.R10-09

Kierończyk B., Kaczmarek S.A., Hejdysz M. et al., 2024. Implementation of a metabolizable energy regression model for black soldier fly larvae fat in broiler chicken diets: effect on growth performance, nutrient digestibility, and selected physiological indices. J. Anim. Feed Sci. 33, 493–503, https://doi.org/10.22358/jafs/187805/2024

Kierończyk B., Rawski M., Mikołajczak Z., Homska N., Jankowski J., Ognik K., Józefiak A., Mazurkiewicz J., Józefiak D., 2022. Available for millions of years but discovered through the last decade: Insects as a source of nutrients and energy in animal diets. Anim. Nutr. 11, 60–79, https://doi.org/10.1016/j.aninu.2022.06.015

Kissoum N., Soltani N., 2016. Spiromesifen, an insecticide inhibitor of lipid synthesis, affects the amounts of carbohydrates, glycogen and the activity of lactate dehydrogenase in *Drosophila melanogaster*. J. Entomol. Zool. Stud. 4, 452–456

Kitto G.B., Briggs M.H., 1962. Lactate dehydrogenase in some insect muscles. Nature 193, 1003–1004, https://doi.org/10.1038/1931003b0

Koto A., Tamura M., Wong P.S. et al., 2023. Social isolation shortens lifespan through oxidative stress in ants. Nat. Commun. 14, 5493, https://doi.org/10.1038/s41467-023-41140-w

Kulma M., Kouřimská L., Homolková D., Božik M., Plachý V., Vrabec V., 2020. Effect of developmental stage on the nutritional value of edible insects. A case study with *Blaberus craniifer* and *Zophobas morio*. J. Food Compos. Anal. 92, 103570, https://doi.org/10.1016/j.jfca.2020.103570

Kulma M., Plachý V., Kouřimská L., Vrabec V., Bubová T., Adámková A., Hučko B., 2016. Nutritional value of three *Blattodea species* used as feed for animals. J. Anim. Feed Sci. 25, 354–360, https://doi.org/10.22358/jafs/67916/2016

Lähteenmäki-Uutela A., Marimuthu S.B.S., Meijer N., 2021. Regulations on insects as food and feed: a global comparison. J. Insects as Food Feed 7, 849–856, https://doi.org/10.3920/JIFF2020.0066

Lam P.Y., Abdul Latif N.S., Thevan K., Rao P.V., Wan Muhamed W.Z., 2021. Nutrient composition of *Blaptica dubia* (Order: Blattodea) as an alternative protein source. J. Trop. Resour. Sustain. Sci. 6, 88–92, https://doi.org/10.47253/jtrss.v6i2.568

Lee K.P., Simpson S.J., Wilson K., 2008. Dietary protein-quality influences melanization and immune function in an insect. Funct. Ecol. 22, 1052–1061, https://doi.org/10.1111/j.1365-2435.2008.01459.x

Litwack G. (Editor), 2022. Metabolism of Amino Acids. Human Biochemistry. Elsevier. Amsterdam (Netherlands), pp. 403–440, https://doi.org/10.1016/B978-0-323-85718-5.00020-0

Liu Z., Zhao J., Lu K., Wang Z., Yin L., Zheng H., Wang X., Mao L., Xing B., 2022. Biodegradation of graphene oxide by insects (*Tenebrio molitor* Larvae): Role of the gut microbiome and enzymes. Environ. Sci. Technol. 56, 16737–16747, https://doi.org/10.1021/acs.est.2c03342

Lou Y., Li Y., Lu B., Liu Q., Yang S.-S., Liu B., Ren N., Wu W.-M., Xing D., 2021. Response of the yellow mealworm (*Tenebrio molitor*) gut microbiome to diet shifts during polystyrene and polyethylene biodegradation. J. Hazard. Mater. 416, 126222, https://doi.org/10.1016/j.jhazmat.2021.126222

Łosiewicz B., Szudrowicz H., 2024. Impact of slaughter method on stress in organic common carp (*Cyprinus carpio*). J. Anim. Feed Sci. 33, 562–570, https://doi.org/10.22358/jafs/189182/2024

Łoś A., Strachecka A., 2018. Fast and cost-effective biochemical spectrophotometric analysis of solution of insect «blood» and body surface elution. Sensors 18, 1494, https://doi.org/10.3390/s18051494

Ma Z., Fu J., Zhang Y., Wang L., Luo Y., 2024. Toxicity and behavior-altering effects of three nanomaterials on red imported fire ants and their effectiveness in combination with indoxacarb. Insects 15, 96, https://doi.org/10.3390/insects15020096

Macedo M.L.R., Freire M. das G.M., 2011. Insect digestive enzymes as a target for pest control. Invertebr. Surviv. J. 8, 190–198

Magsalay A.G., Carnoso C.L., Niepes R.A., 2024. Growth performance, carcass characteristics and meat quality of grower native chicken (*Gallus gallus domesticus* L.) fed with superworm (*Zophobas morio* F.) as protein source substitute to soybean meal (*Glycine max* L.). Livest. Res. Rural Dev. 36, 4

Mali B., Okello S., Ocaido M., Nalule A.S., 2020. Optimal inclusion level of termite meal replacing fish meal in broiler diets. Livest. Res. Rural Dev. 32,11

Marieshwari B.N., Bhuvaragavan S., Sruthi K., Mullainadhan P., Janarthanan S., 2023. Insect phenoloxidase and its diverse roles: melanogenesis and beyond. J. Comp. Physiol. B 193, 1–23, https://doi.org/10.1007/s00360-022-01468-z

Martínez-Girón R., Martínez-Torre C., van Woerden H.C., 2017. The prevalence of protozoa in the gut of German cockroaches (*Blattella germanica*) with special reference to *Lophomonas blattarum.* Parasitol. Res. 116, 3205–3210, https://doi.org/10.1007/s00436-017-5640-6

Matthäus B., Piofczyk T., Katz H., Pudel F., 2019. Renewable resources from insects: exploitation, properties, and refining of fat obtained by cold-pressing from *Hermetia illucens* (black soldier fly) larvae. Eur. J. Lipid Sci. Technol. 121, 1800376, https://doi.org/10.1002/ejlt.201800376

Mba A.R.F., Kansci G., Viau M., Hafnaoui N., Meynier A., Demmano G., Genot C., 2017. Lipid and amino acid profiles support the potential of *Rhynchophorus phoenicis* larvae for human nutrition. J. Food Compos. Anal. 60, 64–73, https://doi.org/10.1016/j.jfca.2017.03.016

Metwally S.A., Abd-Elaziz M.A.A.A.-E., El- Sherif S.I., Ahmed S.S., 2021. Effect of silver and silica nanoparticles on the larvae of pink stem borer *Sesamia cretica* Lederer, 1857 (Lepidoptera: Noctuidae) and maize plants *Zea mays* Linneaus, 1753. Polish J. Entomol. 90, 86–102, https://doi.org/10.5604/01.3001.0014.9174

Miah M.Y., Singh Y., Cullere M., Tenti S., Dalle Zotte A., 2020. Effect of dietary supplementation with full-fat silkworm (*Bombyx mori* L.) chrysalis meal on growth performance and meat quality of Rhode Island Red × Fayoumi crossbred chickens. Ital. J. Anim. Sci. 19, 447–456, https://doi.org/10.1080/1828051X.2020.1752119

Mlček J., Adámková A., Adámek M., Borkovcová M., Bednářová M., Kouřimská L., 2018. Selected nutritional values of field cricket (*Gryllus assimilis*) and its possible use as a human food. Indian J. Tradit. Knowl. 17, 518–524

Mmbone S., Gohole L., Wanjala F., Ronoh A., 2023. Effects of diet on the nutritional composition of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). African J. Trop. Entomol. Res. 2, 1–10, https://doi.org/10.58697/AJTER020101

Moniello G., Ariano A., Panettieri V. et al., 2019. Intestinal morphometry, enzymatic and microbial activity in laying hens fed different levels of a *Hermetia illucens* larvae meal and toxic elements content of the insect meal and diets. Animals 9, 86, https://doi.org/10.3390/ani9030086

Muhammad A., He J., Yu T., Sun C., Shi D., Jiang Y., Xianyu Y., Shao Y., 2022. Dietary exposure of copper and zinc oxides nanoparticles affect the fitness, enzyme activity, and microbial community of the model insect, silkworm *Bombyx mori*. Sci. Total Environ. 813, 152608, https://doi.org/10.1016/j.scitotenv.2021.152608

Nasser R., Ibrahim E., Fouad H., Ahmad F., Li W., Zhou Q., Yu T., Chidwala N., Mo J., 2024. Termiticidal Effects and morpho-histological alterations in the subterranean termite (*Odontotermes formosanus*) induced by biosynthesized zinc oxide, titanium dioxide, and chitosan nanoparticles. Nanomaterials 14, 927, https://doi.org/10.3390/nano14110927

Nath B.S., Suresh A., Varma B.M., Kumar R.P.S., 1997. Changes in protein metabolism in hemolymph and Fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticides toxicity. Ecotoxicol. Environ. Saf. 36, 169–173, https://doi.org/10.1006/eesa.1996.1504

Navarro del Hierro J., Gutiérrez-Docio A., Otero P., Reglero G., Martin D., 2020. Characterization, antioxidant activity, and inhibitory effect on pancreatic lipase of extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor.* Food Chem. 309, 125742, https://doi.org/10.1016/j.foodchem.2019.125742

Nephale L.E., Moyo N.A.G., Rapatsa-Malatji M.M., 2024. Partial replacement of fish meal with soldier termite in juvenile *Mozambique tilapia*: Effects on growth performance, blood serum chemistry and histomorphology. J. Anim. Feed Sci. 33, 243–252, https://doi.org/10.22358/jafs/175919/2024

Oibiokpa F.I., Akanya H.O., Jigam A.A., Saidu A.N., Egwim E.C., 2018. Protein quality of four indigenous edible insect species in Nigeria. Food Sci. Hum. Wellness 7, 175–183, https://doi.org/10.1016/j.fshw.2018.05.003

Okrutniak M., Grześ I.M., 2021. Accumulation of metals in Lasius niger: Implications for using ants as bioindicators. Environ. Pollut. 268, 115824, https://doi.org/10.1016/j.envpol.2020.115824

Orčić S., Nikolić T., Purać J., Šikoparija B., Blagojević D.P., Vukašinović E., Plavša N., Stevanović J., Kojić D., 2017. Seasonal variation in the activity of selected antioxidant enzymes and malondialdehyde level in worker honey bees. Entomol. Exp. Appl. 165, 120–128, https://doi.org/10.1111/eea.12633

Özkan Y., İrende İ., Akdeniz G., Kabakcı D., Sökmen M., 2015. Evaluation of the comparative acute toxic effects of TiO_2, Ag-TiO_2 and ZnO-TiO_2 composite nanoparticles on honey bee (*Apis mellifera*). J. Int. Environ. Appl. Sci. 10, 26–36

Paes M.C., Oliveira M.B., Oliveira P.L., 2001. Hydrogen peroxide detoxification in the midgut of the blood-sucking insect, *Rhodnius prolixus*. Arch. Insect Biochem. Physiol. 48, 63–71, https://doi.org/10.1002/arch.1058

Page M.J., McKenzie J.E., Bossuyt P.M. et al., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst. Rev. 10, 89, https://doi.org/10.1186/s13643-021-01626-4

Park J.-W., Kim M., Kim S.-Y., Bae J., Kim T.-J., 2023. Biodegradation of polystyrene by intestinal symbiotic bacteria isolated from mealworms, the larvae of *Tenebrio molitor*. Heliyon 9, e17352, https://doi.org/10.1016/j.heliyon.2023.e17352

Pedersen K.E., Pedersen N.N., Meyling N.V., Fredensborg B.L., Cedergreen N., 2020. Differences in life stage sensitivity of the beetle *Tenebrio molitor* towards a pyrethroid insecticide explained by stage-specific variations in uptake, elimination and activity of detoxifying enzymes. Pestic. Biochem. Physiol. 162, 113–121, https://doi.org/10.1016/j.pestbp.2019.09.009

Peñaflorida V.D., 1989. An evaluation of indigenous protein sources as potential component in the diet formulation for tiger prawn, *Penaeus monodon*, using essential amino acid index (EAAI). Aquaculture 83, 319–330, https://doi.org/10.1016/0044-8486(89)90043-4

Peng B.-Y., Chen Z., Chen J., Zhou X., Wu W.-M., Zhang Y., 2021. Biodegradation of polylactic acid by yellow mealworms (larvae of *Tenebrio molitor*) via resource recovery: A sustainable approach for waste management. J. Hazard. Mater. 416, 125803, https://doi.org/10.1016/j.jhazmat.2021.125803

Perez-Santaescolastica C., de Pril I., van de Voorde I., Fraeye I., 2023. Fatty acid and amino acid profiles of seven edible insects: focus on lipid class composition and protein conversion factors. Foods 12, 4090, https://doi.org/10.3390/foods12224090

Piechowicz B., Sudoł M., Grodzicki P., Podbielska M., Szpyrka E., Zwolak A., Potocki L., 2021. The dynamics of pyrethroid residues and Cyp P450 gene expression in insects depends on the circadian clock. Environ. Res. 194, 110701, https://doi.org/10.1016/j.envres.2020.110701

Prachom N., Boonyoung S., Hassaan M.S., El-Haroun E., Davies S.J., 2021. Preliminary evaluation of Superworm (*Zophobas morio*) larval meal as a partial protein source in experimental diets for juvenile Asian sea bass, *Lates calcarifer*. Aquac. Nutr. 27, 1304–1314, https://doi.org/10.1111/anu.13269

Puppel K., Slósarz J., Grodkowski G., Solarczyk P., Kostusiak P., Kunowska-Slósarz M., Grodkowska K., Zalewska A., Kuczyńska B., Gołębiewski M., 2022. Comparison of enzyme activity in order to describe the metabolic profile of dairy cows during early lactation. Int. J. Mol. Sci. 23, 9771, https://doi.org/10.3390/ijms23179771

Quinteros M.F., Martínez J., Barrionuevo A., Rojas M., Carrillo W., 2022. Functional, antioxidant, and anti-inflammatory properties of cricket protein concentrate (*Gryllus assimilis*). Biology (Basel). 11, 776, https://doi.org/10.3390/biology11050776

Ramzy A.Y., Seemab Z., Fouzi A.M., 2022. Use of locust meal as alternative protein source to fish meal in practical diets for fingerling *Oreochromis niloticus*. Asian J. Anim. Vet. Adv. 17, 16–27, https://doi.org/10.3923/ajava.2022.16.27

Rocha A.G., Oliveira B.M.S., Melo C.R., Sampaio T.S., Blank A.F., Lima A.D., Nunes R.S., Araújo A.P.A., Cristaldo P.F., Bacci L., 2018. Lethal effect and behavioral responses of leaf-cutting ants to essential oil of *Pogostemon cablin* (Lamiaceae) and its nanoformulation. Neotrop. Entomol. 47, 769–779, https://doi.org/10.1007/s13744-018-0615-6

Saha D., Mukhopadhyay A., Bahadur M., 2012. Effect of host plants on fitness traits and detoxifying enzymes activity of *Helopeltis theivora*, a major sucking insect pest of tea. Phytoparasitica 40, 433–444, https://doi.org/10.1007/s12600-012-0244-2

Salmela H., Sundström L., 2017. Vitellogenin in inflammation and immunity in social insects. Inflamm. Cell Signal. 4, e1506, https://doi.org/10.14800/ics.1506

Santana C.C., Barbosa L.A., Júnior I.D.B., do Nascimento T.G., Dornelas C.B., Grillo L.A.M., 2017. Lipase activity in the larval midgut of rhynchophorus palmarum: biochemical characterization and the effects of reducing agents. Insects 8, 100, https://doi.org/10.3390/insects8030100

Schneider S.A., Schrader C., Wagner A.E., Boesch-Saadatmandi C., Liebig J., Rimbach G., Roeder T., 2011. Stress resistance and longevity are not directly linked to levels of enzymatic antioxidants in the ponerine ant harpegnathos saltator. PLoS One 6, e14601, https://doi.org/10.1371/journal.pone.0014601

Sezer Tuncsoy B., Tuncsoy M., Gomes T., Sousa V., Teixeira M.R., Bebianno M.J., Ozalp P., 2019. Effects of copper oxide nanoparticles on tissue accumulation and antioxidant enzymes of *Galleria mellonella* L. Bull. Environ. Contam. Toxicol. 102, 341−346, https://doi.org/10.1007/s00128-018-2529-8

Sheeja C.C., Anusri A., Levna C., Aneesh P.M., Lekha D., 2020. MoS2 nanoparticles induce behavioral alteration and oxidative stress mediated cellular toxicity in the social insect *Oecophylla smaragdina* (Asian weaver ant). J. Hazard. Mater. 385, 121624, https://doi.org/10.1016/j.jhazmat.2019.121624

Siddiqui S.A., Thanpandiyan K., Adli D.N., Yudhistira B., Fernando I., De Palo P., 2024. Overview of the African palm weevil (*Rhynchophorus phoenicis*) as food and feed - A critical review. J. Insects Food Feed 1, 1–28, https://doi.org/10.1163/23524588-00001005

Simon J.Y., 1996. Insect glutathione s-transferases. Zool. Stud. 35, 9–19

Sioutas G., Tsouknidas A., Gelasakis A.I., Vlachou A., Kaldeli A.K., Kouki M., Symeonidou I., Papadopoulos E., 2023. *In vitro* acaricidal activity of silver nanoparticles (AgNPs) against the poultry red mite (*Dermanyssus gallinae*). Pharmaceutics 15, 659, https://doi.org/10.3390/pharmaceutics15020659

Skaldina O., Sorvari J., 2017. Biomarkers of ecotoxicological effects in social insects. In: U. Forstner, W.H. Rulkens (Editors). Environmental Science and Engineering (Subseries: Environmental Science). Springer Berlin. Heidelberg (Germany), pp. 203–214, https://doi.org/10.1007/978-3-319-46248-6_10

Sogari G., Amato M., Biasato I., Chiesa S., Gasco L., 2019. The potential role of insects as feed: A multi-perspective review. Animals 9, 119, https://doi.org/10.3390/ani9040119

Sujak A., Kotlarz A., Strobel W., 2006. Compositional and nutritional evaluation of several lupin seeds. Food Chem. 98, 711–719, https://doi.org/10.1016/j.foodchem.2005.06.036

Sullivan L.B., Gui D.Y., Heiden M.G. Vander, 2016. Altered metabolite levels in cancer: implications for tumour biology and cancer therapy. Nat. Rev. Cancer 16, 680–693, https://doi.org/10.1038/nrc.2016.85

Szczepański A., Adamek-Urbańska D., Kasprzak R., Szudrowicz H., Śliwiński J., Kamaszewski M., 2022. Lupin: A promising alternative protein source for aquaculture feeds? Aquac. Rep. 26, 101281, https://doi.org/10.1016/j.aqrep.2022.101281

Tasaki E., Sakurai H., Nitao M., Matsuura K., Iuchi Y., 2017. Uric acid, an important antioxidant contributing to survival in termites. PLoS One 12, e0179426, https://doi.org/10.1371/journal.pone.0179426

Teleb S.S., Farag A.A., Mostafa A.A.Z.M., 2012. Effect of pyridalyl on alkaline phosphatase and transaminase activities in some tissues of *Schistocerca gregaria* (Orthoptera: Acrididae). J. Am. Sci. 8, 315–322

Tellis M.B., Kotkar H.M., Joshi R.S., 2023. Regulation of trehalose metabolism in insects: from genes to the metabolite window. Glycobiology 33, 262–273, https://doi.org/10.1093/glycob/cwad011

Tlak Gajger I., Vlainić J., Šoštarić P., Prešern J., Bubnič J., Smodiš Škerl M.I., 2020. Effects on some therapeutical, biochemical, and immunological parameters of honey bee (*Apis mellifera*) exposed to probiotic treatments, in field and laboratory conditions. Insects 11, 638, https://doi.org/10.3390/insects11090638

Tomé H.V.V., Schmehl D.R., Wedde A.E., Godoy R.S.M., Ravaiano S. V., Guedes R.N.C., Martins G.F., Ellis J.D., 2020. Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. Environ. Pollut. 256, 113420, https://doi.org/10.1016/j.envpol.2019.113420

Toprak U., Hegedus D., Doğan C., Güney G., 2020. A journey into the world of insect lipid metabolism. Arch. Insect Biochem. Physiol. 104, e21682, https://doi.org/10.1002/arch.21682

Tufail M., Nagaba Y., Elgendy A.M., Takeda M., 2014. Regulation of vitellogenin genes in insects. Entomol. Sci. 17, 269–282, https://doi.org/10.1111/ens.12086

Turek J., Sampels S., Khalili Tilami S. et al., 2020. Insects in the feed of rainbow trout *Oncorhynchus mykiss* (Actinopterygii, Salmonidae): effect on growth, fatty acid composition and sensory attributes. Acta Ichthyol. Piscat. 50, 171–181, https://doi.org/10.3750/AIEP/02785

van Huis A., 2020. Insects as food and feed, a new emerging agricultural sector: a review. J. Insects as Food Feed 6, 27–44, https://doi.org/10.3920/JIFF2019.0017

van Huis A., van Itterbeck J., Klunder H., Mertens E., Halloran A., Muir G., Vantomme P., 2013. Edible insects: future prospects for food and feed security. Food and Agriculture Organisation of the United Nations. Rome (Italy)

Venugopal V., Gopakumar K., 2017. Shellfish: nutritive value, health benefits, and consumer safety. Compr. Rev. Food Sci. Food Saf. 16, 1219–1242, https://doi.org/10.1111/1541-4337.12312

Vrabec V., Kulma M., Cocan D., 2015. Insects as an alternative protein source for animal feeding: a short review about chemical composition. Bull. Univ. Agric. Sci. Vet. Med. Cluj-Napoca. Anim. Sci. Biotechnol. 72, 116–126, https://doi.org/10.15835/buasvmcn-asb:11656

Vural O., Tunç A., Kamaszewski M., Aksakal E., 2023. Is royal jelly a sustainable alternative lipid source in aquaculture? Influence of dietary royal jelly levels on fatty acid composition in zebrafish. J. Anim. Feed Sci. 32, 459–467, https://doi.org/10.22358/jafs/161997/2023

Wang G., Zhou J.-J., Li Y., Gou Y., Quandahor P., Liu C., 2021. Trehalose and glucose levels regulate feeding behavior of the phloem-feeding insect, the pea aphid *Acyrthosiphon pisum* Harris. Sci. Rep. 11, 15864, https://doi.org/10.1038/s41598-021-95390-z

Wangberg H., Mendoza J., Gomez R., Coop C., White A., Woessner K., 2021. The first reported case of *Blaptica dubia* cockroach allergy. Allergy Asthma Clin. Immunol. 17, 114, https://doi.org/10.1186/s13223-021-00618-2

Weihrauch D., O'Donnell M.J., 2021. Mechanisms of nitrogen excretion in insects. Curr. Opin. Insect Sci. 47, 25–30, https://doi.org/10.1016/j.cois.2021.02.007

Wu Z., Yang L., He Q., Zhou S., 2021. Regulatory mechanisms of vitellogenesis in insects. Front. Cell Dev. Biol. 8, 593613, https://doi.org/10.3389/fcell.2020.593613

Xiong T., Ling S., Liu J., Zeng X., 2022. Insecticidal and P450 mediate metabolism of fluralaner against red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Pestic. Biochem. Physiol. 187, 105184, https://doi.org/10.1016/j.pestbp.2022.105184

Xiong T., Qiu X., Ling S., Liu J., Zeng X., 2019. Interaction of fipronil and the red imported fire ant (*Solenopsis invicta*): Toxicity differences and detoxification responses. J. Insect Physiol. 115, 20–26, https://doi.org/10.1016/j.jinsphys.2019.03.001

Yamada T., Habara O., Kubo H., Nishimura T., 2018. Fat body glycogen serves as a metabolic safeguard for the maintenance of sugar levels in *Drosophila*. Development 145, dev158865, https://doi.org/10.1242/dev.158865

Yang L., Chen H., Zheng Q., Luo P., Yan W., Huang S., Cheng D., Hong Xu H., Zhang Z., 2023. A β-cyclodextrin-functionalized metal-organic framework enhances the insecticidal activity of indoxacarb by affecting amino acid metabolism in red imported fire ants. Chem. Eng. J. 458, 141417, https://doi.org/10.1016/j.cej.2023.141417

Yang S.-S., Brandon A.M., Andrew Flanagan J.C. et al., 2018. Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* Linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. Chemosphere 191, 979–989, https://doi.org/10.1016/j.chemosphere.2017.10.117

Yi S., Adams T.S., 2001. Age- and diapause-related acid and alkaline phosphatase activities in the intestine and malpighian tubules of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Arch. Insect Biochem. Physiol. 46, 152–163, https://doi.org/10.1002/arch.1025

Zebe E.C., McShan W.H., 1957. Lactic and α-glycerophosphate dehydrogenases in insects. J. Gen. Physiol. 40, 779–790, https://doi.org/10.1085/jgp.40.5.779

Zheng Q., Wang R., Qin D., Yang L., Lin S., Cheng D., Huang S., Zhang Z., 2021. Insecticidal efficacy and mechanism of nanoparticles synthesized from chitosan and carboxymethyl chitosan against *Solenopsis invicta* (Hymenoptera: Formicidae). Carbohydr. Polym. 260, 117839, https://doi.org/10.1016/j.carbpol.2021.117839

Zibaee A., Zibaee I., Jalali Sendi J., 2011. A juvenile hormone analog, pyriproxifen, affects some biochemical components in the hemolymph and fat bodies of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). Pestic. Biochem. Physiol. 100, 289–298, https://doi.org/10.1016/j.pestbp.2011.05.002

Zielińska E., Baraniak B., Karaś M., Rybczyńska K., Jakubczyk A., 2015. Selected species of edible insects as a source of nutrient composition. Food Res. Int. 77, 460–466, https://doi.org/10.1016/j.foodres.2015.09.008