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LETTER TO THE EDITOR

Knowledge gaps in feeding physiology, microbiome and behaviour of insects for food and feed: overcoming barriers to advancing insect-rearing through interdisciplinarity, standardisation, and emerging technologies

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Abstract

The mass rearing of insects for food and feed has emerged as a promising solution to food insecurity, protein production and waste valorisation. While outcomes such as growth and feed conversion efficiency have been examined for mass reared insects, the mechanisms underpinning these outcomes are poorly understood. To optimise the rearing of insects for food and feed and build towards efficient, scalable and productive systems, we must first therefore understand the mechanisms driving productivity and efficiency in common target species. By integrating emerging technologies across disciplines, we can begin to characterise these mechanisms to guide the optimisation of robust and standardised systems for efficient and scalable food and feed production. In this article, we explore opportunities for integrated and interdisciplinary thinking, and the use of emerging technologies to characterise the feeding physiology, digestive processes, microbiome-driven nutritional effects and behavioural regulation of insects commonly used for food and feed. We identify areas of synergy and interaction across research domains and provide a roadmap and call to action to catalyse uptake of these ideas across research and practice. By realising interdisciplinary integration and implementation of emerging technologies, we believe that an urgently

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required deeper mechanistic understanding of feeding-related processes is possible. This can, in turn, enable precision nutrition, improve system robustness and ensure consistent product quality.

Keywords

Hermetia illucens – *Tenebrio molitor* – *Acheta domesticus* – microbiome-nutrition interactions – digestive physiology – feeding behaviour

1 Introduction

As global demand for food and feed continues to increase, concerns regarding affordability, sustainability and resource efficiency have intensified, promoting renewed interest in non-traditional sources (FAO, 2023; Malila *et al.*, 2024). Mass production of insect species has emerged as a promising strategy to address challenges related to food security, sustainable protein production and organic waste valorisation (van Huis, 2013). Species such as *Hermetia illucens* L. (black soldier fly, BSF) (Diptera: Stratiomyidae), *Tenebrio molitor* L. (yellow mealworm) (Coleoptera: Tenebrionidae), and *Acheta domesticus* L. (house cricket) (Orthoptera: Gryllidae) are increasingly produced on an industrial scale due to their feed conversion efficiency, small footprint and high nutritional value (Gasco *et al.*, 2020; Oonincx and Finke, 2021; van Huis, 2013). Despite this growing interest, fundamental knowledge of the feeding behaviour, digestive physiology, and microbiome-driven nutritional effects of these insects remains limited, especially when compared with conventional livestock systems (Jordan and Tomberlin, 2021; Rho and Lee, 2022; Tettamanti *et al.*, 2022). For non-model insects, and particularly insects reared on an industrial scale, most studies have focused on performance parameters such as growth and feed conversion efficiency (Pinotti and Ottoboni, 2021; Radish and Akmali, 2025; Sandrock *et al.*, 2025), while the mechanistic processes affecting these outcomes remain largely unexplored. This knowledge gap constrains the development of species-specific feeding strategies and limits the efficiency, scalability and productivity of insect-rearing systems due to sub-optimal feed intake, poor nutrient utilisation, variable growth and reduced production predictability on an industrial scale.

Insects exhibit diverse nutritional strategies, gastrointestinal morphologies and behavioural adaptations to feeding traits; however, beyond a few model species, such as the fruit fly *Drosophila melanogaster*, the red flour beetle *Tribolium castaneum*, and the greater wax moth *Galleria mellonella* (Miguel-Aliaga *et al.*, 2018; Tonk-Rugen *et al.*, 2022), these traits remain insufficiently characterised. For industrially relevant insects, such as BSF, mealworms and crickets, a deeper mechanistic understanding of feeding-related processes is urgently needed to enable precision nutrition, improve system robustness, and ensure consistent product quality.

In this article, we argue that progress in insect-rearing research critically depends on advances across three interconnected domains (Figure 1): (1) microbiome-nutrition interactions, (2) feeding behaviour and (3) digestive physiology. For each domain, we highlight key knowledge gaps, methodological biases and interdisciplinary approaches that could accelerate innovation in insect-rearing systems for food and feed production. By summarising these gaps, biases and opportunities, we hope to catalyse increased effort towards advancing insect rearing for the establishment of a new generation of efficient, scalable and productive food and feed production systems.

2 Microbiome–nutrition interactions

The insect symbiome, encompassing bacteria, fungi, viruses, protozoa and endosymbionts, plays a pivotal role in nutrient hydrolysis and assimilation, immune function and metabolic adaptation (Douglas, 2009; Wilder *et al.*, 2025). While nutritional symbioses have been extensively studied in termites (Bourguignon *et al.*, 2018), aphids (Feng *et al.*, 2019) and certain beetles (Salem *et al.*, 2023), their functional relevance for mass-reared insects remains poorly understood, as basic methodological and conceptual gaps complicate the interception of the microbiome effect on insect nutrition. In species such as *H. illucens* and *T. molitor*, which are reared in constant proximity to their feed substrate, both gut and environmental microbial communities are known to respond strongly to diet composition and rearing conditions (Eke *et al.*, 2023; Montalbán *et al.*,

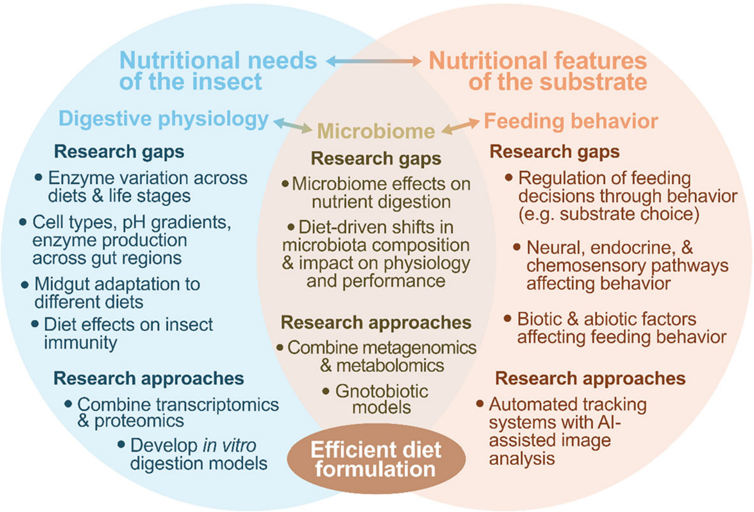

FIGURE 1 Main gaps and approaches required for advancing innovation in insect-rearing systems for food and feed production.

2022), yet their direct contribution to host nutrition and performance is still largely unclear. Studies focused on housefly (*Musca domestica*) and BSF larvae indicate that the presence of microbes is often required for normal development (Auger *et al.*, 2023; Pei *et al.*, 2023; Zurek *et al.*, 2000); however, in most species it remains unresolved whether associated microbes primarily act as providers of energy and essential nutrients (e.g. through fermentation of complex polysaccharides), competitors for dietary resources or opportunistic passengers which may become beneficial or harmful under some environmental conditions. It is likely that these roles are highly taxon- and context-dependent, but a deeper understanding is required to predict and characterise which taxa and contexts alter outcomes. Moreover, non-bacterial components of the (gut) microbiome, particularly fungi, are increasingly recognised as potentially important contributors to digestion, nutrient availability, and metabolic signalling (Ben-Mordechai *et al.*, 2025), yet remain underrepresented in most insect-related studies.

A central limitation of current research is the predominance of descriptive, composition-based approaches, largely relying on 16S rRNA gene amplicon sequencing. While these methods provide valuable taxonomic insights, they offer limited information on absolute abundance, organism viability, metabolic activity or functional relevance (Bruno *et al.*, 2019; Weinroth *et al.*, 2022). Many of these data can be provided by alternative analytical methods, with approaches like metatranscriptomics (the analysis of gene expression across multiple organisms by analysing mRNA) and metabolomics (the analysis of metabolites present in a sample) providing functional insights into microbial communities. Integrative approaches combining metagenomics, metatranscriptomics, metabolomics and stable isotope tracing are therefore essential to quantify microbial contributions to digestion and host metabolism (Auger *et al.*, 2025; Ijdema *et al.*, 2025). Moreover, the development of axenic (free of microbes) and gnotobiotic (with all microbes known) insect models is crucial for studying host-microbe interactions, disentangling host-driven and microbiome-driven nutritional, metabolomic and immune processes, and for testing causal hypotheses under controlled conditions (Wu *et al.*, 2023). By determining the functional roles of different microbial taxa in insect nutrition and development, they could be managed (e.g. using targeted microbial depletion; Du Toit, 2020) to optimise feed conversion efficiency and growth.

Another research gap concerns the dynamic nature of microbiome-nutrition interactions. Most current studies rely on single time points, overlooking temporal changes in substrate composition and associated shifts in the environmental microbiome, as well as insect developmental stages, moulting events, and feeding cycles, which may critically influence digestive efficiency and nutrient allocation (McMullen *et al.*, 2020). Likewise, microbial functions can differ markedly along the gut because each section has distinct pH conditions and is specialised for processes such as fermentation or nutrient absorption, making it difficult to determine the microbiome's overall role in digestion (Mondal *et al.*, 2023). In addition, environmental microbiota associated with substrates, frass, and rearing equipment may perform important external digestive functions while acting only as transient community members in the insect gut, challenging the distinction between resident symbionts and environmental contaminants (Ravenscraft and Coon, 2025). Finally, the ecological and evolutionary drivers shaping host-microbiome associations in reared insects are not well characterised. Comparative studies across phylogenetically distant species or rearing systems could reveal whether microbiome-mediated nutritional traits are conserved, convergent, or shaped by artificial selection during domestication. Understanding these processes will be vital for leveraging microbiota to enhance feeding efficiency, resilience and product quality in sustainable insect farming systems.

Key research questions that should be further studied in this topic include:

(1) What are the quantitative contributions of microorganisms to nutrient assimilation across diets and developmental stages?
(2) How do microbiome interactions influence digestive efficiency and production performance?
(3) Can microbiome manipulation (e.g. probiotics, prebiotics, selective depletion) be implemented to enhance feed conversion, robustness and product quality in mass-reared insects (Jordan and Tomberlin, 2021; Savio *et al.*, 2022; Schreven *et al.*, 2021)?

Addressing these questions requires not only interdisciplinary methodological advancements, such as the use of metagenomics, metatranscriptomics and metabolomics, but also standardised experimental frameworks that enable cross-species comparisons and reproducible, targeted interventions on the microbiome in insect-rearing systems.

3 Determinants of feeding behaviour

Insect feeding behaviour is a central determinant of nutrient intake, digestion and growth (Cuff *et al.*, 2024a); however, in the context of insect rearing, this behavioural complexity is often oversimplified (Ruedenauer *et al.*, 2023; Tomberlin *et al.*, 2025). Insects integrate sensory cues, internal nutritional state, microbial signals and environmental conditions to evaluate and exploit food resources (Wilder *et al.*, 2025). Olfactory and gustatory systems enable them to detect volatiles and contact cues associated with nutrient availability (Joseph and Carlson, 2015; Leitão-Gonçalves *et al.*, 2017; Robertson *et al.*, 2019), while mechanosensory feedback, related to moisture, texture and particle-size perception further modulate feeding decisions (Freeman *et al.*, 2014; Zhou *et al.*, 2019).

Internal nutritional state dynamically modulates sensory perception and feeding motivation. Nutrient-deprived individuals show increased sensitivity to previously neutral or even aversive chemical cues (Corrales-Carvajal *et al.*, 2016; Steck *et al.*, 2018); for example, essential amino acid deficiency modulates olfactory and gustatory receptor gene expression, increasing behavioural attraction towards microbe-rich or protein-containing substrates (Ezra-Nevo *et al.*, 2025). Whether such mechanisms generalise across industrially reared species, developmental stages and group-rearing conditions remains largely unknown.

Microbial activity within substrates further complicates feeding behaviour by altering environmental chemical prevalence and releasing volatile organic compounds (VOCs) that modulate insect foraging and acceptance behaviours (Davis *et al.*, 2013; Klammsteiner *et al.*, 2025a), depending on fermentation stage and microbial community structure. These microbial VOCs can enhance palatability or, conversely, signal spoilage or toxicity depending on composition and fermentation stage (Davis *et al.*, 2013; Leitão-Gonçalves *et al.*, 2017).

There is also a limited understanding of how environmental factors such as population density, temperature or light influence feeding patterns and social interactions in these inherently group-reared systems (Kortsmit *et al.*, 2023). In *H. illucens*, for example, aggregate larval activity appears to vary significantly over the course of rearing (Kortsmit *et al.*, 2023), but it is unclear whether this behaviour is evolutionarily hard-coded or occurs in response to specific conditions. Density-dependent larval thermogenesis (i.e. the increased generation of heat by densely populated larvae) has long been acknowledged as a source of system dysbiosis (i.e. imbalance or instability of the rearing system) in large-scale rearing and has broad impacts on physicochemical and microbiological dynamics (Klammsteiner *et al.*, 2025b).

In mass-rearing contexts, feeding behaviour is most often inferred directly from feed intake and growth outcomes, while factors such as the mouthpart morphology, substrate accessibility or behavioural constraints on ingestion are rarely examined explicitly as mechanisms shaping feeding. Moreover, mechanical barriers (e.g. texture, particle size), non-digestible components (e.g. fibres, anti-nutrients), chemical cues (e.g. palatable compounds), environmental factors (e.g. temperature), social interactions and density-dependent effects are often managed in rearing systems but are less often manipulated nor examined as factors shaping feeding decisions and ingestion efficiency. Such simplifications fail to reflect the species-specific behavioural ecology of reared insects and conspecifics interactions, which can lead to suboptimal diet formulations and management strategies, particularly in systems where feeds are deliberately designed to maximise production efficiency, such as insect rearing for food and feed. Together, these dynamics are particularly important in mass-rearing facilities, where behavioural variation can lead to uneven resource access, reduced performance or increased mortality. Recent studies have highlighted the complexity of these parameters in designing mass-rearing protocols for edible insects, which may significantly impact biomass production on a commercial scale (Athanassiou *et al.*, 2024; Coudron *et al.*, 2025).

Key research questions that should be further studied in this topic include:

(1) Which neural, endocrine, and chemosensory mechanisms regulate feeding behaviour in mass-reared insects, and how do these mechanisms influence feed acceptance, intake and foraging decisions under rearing conditions?
(2) To what extent do microbial-derived cues shape feeding motivation and synchronisation?
(3) How can behavioural insights be leveraged to design substrates and rearing conditions that reduce variation in feeding among individuals, thereby promoting uniform growth, resource conversion and overall rearing efficiency?
(4) How does density-dependence influences feeding-related behaviours?

To address these gaps, behavioural research in insect-rearing systems must develop beyond direct observations, as these can introduce biases and poorly reflect the interactions that insects engage in within their food environment when unobserved (Cuff *et al.*, 2022). Automated tracking systems (Plum, 2024), AI-assisted image analysis (Suresh *et al.*, 2024), and controlled behavioural assays can offer high-throughput, quantitative insights into feeding dynamics at both the individual and group levels. Additionally, interdisciplinary collaborations across neuroethology, animal behaviour and computational modelling as well as the use of approaches such as transcriptomics, can significantly advance our ability to decode and predict insect feeding responses. Such knowledge can then be integrated into population dynamics models, allowing a better understanding of density-dependent or numerical effects in reared species.

4 Digestive physiology

Digestive physiology determines the efficiency with which insects extract energy and nutrients from resources, yet it remains comparatively underexplored in species reared for food and feed. Unlike conventional livestock systems, where digestive systems are extensively mapped and manipulated to improve productivity (Beever, 1992), insect digestion is often studied in a taxonomically narrow and descriptive manner (Holtof *et al.*, 2019). This limits our ability to translate physiological knowledge into predictive feeding strategies for mass-reared insects.

Insects exhibit diverse digestive strategies that rely on endogenous enzymes, physiochemical gradients and, in some cases, symbiotic relationships to digest complex substrates (Caccia *et al.*, 2025; Calderón-Cortés *et al.*, 2012). The insect gut is a highly structured and dynamic system, in which spatial and temporal organisation, including variation in pH, redox potential, enzyme distribution and activity, and tissue specialisation, significantly impact digestive processes but are rarely quantified in applied rearing systems. Evidence from Tenebrionid beetles and cockroaches demonstrates pronounced gut compartmentalisation that supports distinct enzymatic activities (Day and Powning, 1949; Vinokurov *et al.*, 2009), suggesting that digestive efficiency cannot be inferred from bulk measurements alone; however, such fine-scale physiological resolution is rarely incorporated into applied insect-rearing research.

In *H. illucens* larvae, the midgut exhibits high functional plasticity, which facilitates processing of low-quality and variable substrates (Bonelli *et al.*, 2020; Bruno *et al.*, 2025a). Morphological and physiological adaptations of the midgut have been linked to dietary shifts (Bruno *et al.*, 2019; Bruno *et al.*, 2025b), yet how host-derived enzymes versus microbial metabolism contribute to substrate breakdown remains unresolved. In addition, external digestion through oral secretion of enzymes as a digestive adaptation and behavioural strategy has been hypothesised to increase substrate breakdown but remains poorly documented in mass-reared insects (Guillaume *et al.*, 2024). In detritivorous insects such as *H. illucens* and *T. molitor*, substrate decomposition/fermentation is also likely to play an important role. There is currently a critical gap in our understanding of how these mechanisms interact and how they respond to extreme or fluctuating feeding conditions.

Digestive physiology is further shaped by interactions between insects and their gut microbiota, which can vary substantially across developmental stages, diets and insect taxa (Gohl *et al.*, 2022; Huang *et al.*, 2021). For example, in the moth *Brithys crini*, distinct life stages harbour different microbial communities that contribute to digestion, nutrition and detoxification (González-Serrano *et al.*, 2020). Across insects, microbial contributions to digestive efficiency, pathogen protection and metabolic regulation range from minimal to essential (Schmidt and Engel, 2021). These interactions are highly context-dependent and influenced by environmental conditions, social behaviour and evolutionary history, underscoring the need to integrate microbiome dynamics into predictive and explanatory models of insect digestion (Ponton *et al.*, 2023).

Despite these complexities, most studies of insect digestion rely on static or end-point measurements, such as growth performance or bulk enzyme activity. By contrast, livestock research routinely integrates *in vivo* and *in vitro* digestion models to predict nutrient availability and guide diet formulation (Bornhorst *et al.*, 2016). The absence of comparable multiscale and quantitative frameworks in insect systems represents a major bottleneck for rational diet design, particularly for species with distinct gut architectures and enzymatic profiles (Seyedalmoosavi *et al.*, 2022).

Beyond nutrient acquisition, digestive physiology is closely linked to immune function and stress resilience. Nutritional immunology approaches, widely applied in livestock, remain underdeveloped in insects, despite evidence that diet composition and gut physiology strongly influence resistance to pathogens and environmental stressors (Ponton *et al.*, 2023; Singer *et al.*, 2014; Xu *et al.*, 2022). In mass-rearing facilities, cross-infestation and disease outbreaks can cause severe quantitative losses and qualitative degradations (Deruytter *et al.*, 2021), and dietary modulation of digestive-immune interactions may offer an effective, yet underutilised, mitigation strategy. Importantly, these approaches must also consider food and feed safety, as digestive processes influence the persistence or elimination of biological and chemical hazards relevant to consumers (Hubert *et al.*, 2018).

Progress in understanding insect digestive physiology is further constrained by methodological fragmentation. The absence of standardised methodologies for measuring digestive efficiency, enzyme kinetics and gut microbiome functionality across species hinders data comparability, meta-analytical synthesis and cross-study comparisons. Moreover, digestion is a dynamic process influenced by environmental variables such as temperature, humidity, and substrate composition, yet real-time or non-invasive monitoring tools are still rarely applied. Integrating approaches such as isotopic tracing, biosensors, transcriptomics, proteomics, and *in vivo* enzymatic assays, already emerging in *H. illucens*, *T. molitor* and other edible insects, offers a path toward mechanistic and predictive models of digestion under realistic rearing conditions.

Key research questions that should be further studied in this topic include:

(1) How does enzymatic activity vary across diets, developmental stages, and rearing conditions?
(2) What are the relative contributions of gastrointestinal compartments, cell types, physicochemical gradients, host enzymes, and microbial metabolism to substrate breakdown?
(3) How have digestive traits co-evolved with dietary specialisation and microbial associations?

Addressing these questions is essential for the development of predictive *in vitro* digestion models and precision feeding strategies for mass-reared insects (Gold *et al.*, 2020). Advancing insect digestive physiology from a descriptive to a precisely quantitative applied science will require an interdisciplinary framework that bridges insect physiology, microbiology, nutritional biochemistry and systems biology. Such integration is essential for developing precision feeding strategies, improving robustness and sustainability and enabling the scalable production of insects as food and feed.

5 Future directions and call to action

Bridging the knowledge gaps in insect microbiome-nutrition interactions, feeding behaviour, and digestive physiology is essential to unlocking the full potential of insect-rearing systems. These domains are inherently interconnected: the microbiome shapes nutrient availability and digestive processes; digestion influences physiological state and immune function; and feeding behaviour mediates exposure to both nutrients and microbes. Progress therefore requires integrative research that explicitly addresses these feedbacks rather than treating each domain separately.

To move beyond empirical diet optimisation and toward predictive, species-specific nutritional models, the field must adopt interdisciplinary strategies that combine insect physiology, microbiology, behavioural ecology, nutritional biochemistry, engineering and computational modelling. Emerging technologies, including high-throughput multi-omics, automated behavioural monitoring, computer vision, artificial intelligence and systems biology provide unprecedented opportunities to generate mechanistic and predictive insights. Realising this potential will, however, depend on coordinated efforts to standardise experimental designs, data reporting and performance metrics across species, life stages and rearing systems (Bosch *et al.*, 2020).

Community-driven initiatives and collaborative networks, such as those emerging from international consortia and COST Actions (e.g. GIN-TONIC, group on insect nutrition: to open nutritional innovative challenges; CA23127), play a critical role in aligning research priorities, sharing protocols, and fostering dialogue between academia and industry (Cuff *et al.*, 2024b). Dedicated workshops, symposia and benchmarking studies can accelerate consensus-building and facilitate the translation of fundamental discoveries into applied solutions for insect production. To catalyse these interactions, the availability of transdisciplinary funding opportunities and the accessibility of relevant networks will be paramount. Broader engagement of researchers across traditional disciplinary boundaries will, however, be necessary to develop common terminology and understanding through which cross-pollination of ideas, methods and practices can truly advance the frontier of insect rearing and nutrition.

A parallel priority is the integration of insect-welfare considerations into feeding and rearing research. As evidence accumulates that nutrition, microbiome composition and environmental conditions influence stress responses and survival, future rearing protocols must be designed with both productivity and welfare in mind (Barrett and Fischer, 2023; Crump *et al.*, 2023). Anticipating regulatory developments and societal expectations in this area will ensure the long-term viability and acceptance of insect-farming systems.

6 Conclusions

Significant progress is required to elucidate the role of insect microbiomes, physiology and behaviour in insect rearing and production. We call for increased collaboration across disciplines, including entomology, microbiology, behavioural ecology, computational modelling and engineering, to investigate and design improved rearing systems for insects. The increased adoption of established and emerging technologies will be crucial to this endeavour, alongside standardisation of current research practices to safeguard best practice. Funding agencies and policy makers should prioritise research that advances not only rearing technologies but also the foundational science behind insect nutrition, particularly in non-model, industrially relevant species. Considering that the mass production of insects for food and feed is still a 'grey area' in terms of its adoption by the wider public of many countries, additional work is required to prioritise more targeted communication strategies, emphasising the environmental benefits of this sector. Developing a robust knowledge base will accelerate innovation in insect-based food systems, reduce resource use and support sustainable agriculture and circular bioeconomy goals. These priorities will ensure a responsible and scientifically robust advancement and expansion of insect rearing.

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