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RESEARCH ARTICLE

BugBook: Data analysis methods in studies of insects for food and feed

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Abstract

In recent decades, research on insect production for food and feed has expanded significantly, driven by advances in farming, processing, genetics, and sustainability. Various data analysis methods, from traditional statistics to advanced machine learning, are used to optimise aspects of insect-based systems. In production, methods like analysis of variance (ANOVA) and regression analysis help improve breeding conditions and growth rates, while multivariate analyses support processing studies by evaluating nutritional and microbial safety. Genetic research leverages bioinformatics, genome-wide association studies (GWAS), and quantitative genetics to enhance traits like yield and disease resistance. Sustainability assessments use life cycle analysis (LCA) with Monte Carlo simulations to measure environmental impacts. Emerging tools, such as neural networks and support vector machines, are gaining traction for predicting feed conversion ratios and disease detection. Despite progress, a comprehensive guide that bridges classic and novel data analysis methods in insect research is still lacking. This study aims to address this gap by offering an accessible manual for researchers and professionals. It will consolidate methodologies across disciplines, highlighting foundational tools for beginners while showcasing advanced techniques for experts. Topics include the application of tailored methods like chitin and protein accounting, sensory analysis, consumer preference modelling, and data visualisation to improve stakeholder communication. By enhancing methodological rigor and fostering transparency, this guide will promote innovation, facilitate data interchange, and ensure the replicability of findings. Ultimately, it aims to drive sustainable advancements in the mass production of insects for food and feed.

Keywords

data analysis methods – genetic research – insect production – machine learning – sustainability assessment

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1 Introduction

In the last few decades, research in insect production for food and feed has grown more than ten-fold (van Huis, 2020), reflecting ongoing rapid academic and applied progress in the sector (Athanassiou *et al.*, 2024). Studies related to the different fields of insect farming, processing, genetics, and sustainability have relied on both standard statistical analysis methods, as well as on more advanced methods exploiting deep learning, behavioural analysis and sustainability assessment. Most studies rely on standard data analysis methods used in related fields. For example, in studies of insect production and utilisation, data analysis methods span a broad spectrum from traditional statistical techniques to cutting-edge machine learning algorithms. In production, methods such as analysis of variance (ANOVA) and regression analysis are pivotal for optimising breeding conditions and growth rates (Deruytter *et al.*, 2019; Rumbos *et al.*, 2020). Processing studies often employ multivariate analysis to assess the impact of various treatments on nutritional content and microbial safety (Vandeweyer *et al.*, 2017; Vanqa *et al.*, 2022). Genetic research also benefits from multivariate analyses (Generalovic *et al.*, 2023; Kaya *et al.*, 2021), but particularly from dedicated bioinformatics tools (Moretta *et al.*, 2020) and genome-wide association studies (GWAS) (Eriksson and Picard, 2021) to annotate genomes and gene functions (Devi *et al.*, 2016; Generalovic *et al.*, 2021), with heritability of desired traits then being evaluated and harnessed by means of adequate phenotyping pipelines (Laursen *et al.*, 2021) quantitative genetics approaches to select desirable traits (Hansen *et al.*, 2024) that enhance yield, disease resistance and product quality (Pal *et al.*, 2024). The various concepts and approaches used for genetics of insects for food and feed are covered in-depth in Sandrock *et al.* (2025). Sustainability assessments utilise life cycle assessment (LCA) approaches with Monte-Carlo Simulation analysis to quantify the significance of environmental impacts of insect farming (Roffeis *et al.*, 2020; Smetana *et al.*, 2019; Tello *et al.*, 2021). Machine learning algorithms, including neural networks and support vector machines, are being increasingly researched for predicting and improving feed conversion ratios and for detecting diseases (Baur *et al.*, 2022; Kyalo *et al.*, 2024; Majewski *et al.*, 2022, 2024). Insect-based feed and food products are supported by sensory analysis and consumer preference modelling (Mishyna *et al.*, 2020; van Huis and Rumpold, 2023). By integrating these diverse methods, researchers can address the multifaceted challenges of insect-based systems, thereby driving innovations that make insect farming more viable and sustainable. Moreover, several studies highlight the need for more specialised data analysis methods that account for the unique aspects of insect research in food and feed (Hansen *et al.*, 2024; Nawoya *et al.*, 2024; Snart *et al.*, 2015). Chitin and protein accounting methods are excellent examples of adaptations for insect studies (Janssen *et al.*, 2017; Luparelli *et al.*, 2023). These methodologies serve as exemplary tools in the nitrogen methods, effectively reducing the overestimation of protein content in insects. Therefore, the current literature lacks a consolidated guide that bridges classic and novel data analysis methods specific to mass production of insects for food and feed.

Researchers and industry professionals often struggle to choose the right methodologies for genetics, sustainability, and product development. This study aimed to bridge the gap between traditional and modern data analysis methods by systematically evaluating and integrating approaches used across diverse disciplines in insect-related research for food and feed. Specifically, it provided a structured framework for understanding data analysis techniques, catering to both beginners and experts in the field. For newcomers, the study offered a comprehensive overview of fundamental methods, establishing a solid foundation for their application. At the same time, it highlighted cutting-edge analytical tools, showcasing their potential for advancing research and innovation in the field. By serving as a guiding manual, this publication should enhance methodological rigor, foster transparency, and promote data comparability. Ultimately, it seeks to facilitate the adoption of robust and replicable research practices, ensuring the continued development of insect-related studies in both academic and industrial contexts.

2 Experimental design in insect studies

Experimental design principles

If we provide a larva with a specific feed and it grows well, can we conclude that this feed is the optimal choice to maximise its performance? It is possible that the growth rate would have been higher with another diet. It is essential to have at least two treatments: one larva should be given a control feed, while the other larva should be given the test target. However, if the larva grows more with the feed to be tested, the result would not be particularly noteworthy. It could be attributed to chance or because the larva has superior genetics. The first principle of experimentation is replication. To obtain reliable results, it is essential to test many larvae with both the control and the treatment. Replication allows us to have a representative sample of the population and reduces the effect of individual variability, increasing the power of the statistical analysis (Morris, 2006).

Before starting any experiment, it is essential to ascertain the minimum number of replicates for each treatment. This will ensure a representative sample and help identify significant differences (at least at $P < 0.05$) for relevant trait changes. The number of replicates can be readily determined using the formula for the least significant differences (d):

$$d = t\sqrt{2}\sqrt{\left(s^2/\mathrm{n}\right)}; \quad \mathrm{n} = 2t^2\mathrm{CV}^2/d\%^2$$

where s^2 is the variance of the error, and CV and $d\%$ are the coefficient of variation and the difference to be found both in percentage. For instance, Pascual *et al.* (2024) conducted a growth experiment with yellow mealworms using 12 crates per treatment and obtained CV values of 7 and 14% for the growth and feed intake parameters, respectively. Clearing $d\%$ from the previous formula ($d\% = \sqrt{2}t\mathrm{CV}/\sqrt{\mathrm{n}}$) and considering a t of 1.8 for a n of 12, only differences among means ($d\%$) greater to 5 and 10% in weight and intake, respectively, could be identified as significant ($P < 0.05$).

If I were to inform you that the smallest larvae have been allocated to the control treatment and the largest ones to the study treatment, what would be your initial reaction? A second key principle is randomisation. It is essential to randomly allocate the experimental units (larvae, adult insects, crates, etc.) across the different treatments. Treatments should be concealed from operators, but the distribution of experimental units in growing or breeding rooms should follow a structured pattern. Environmental conditions (temperature, relative humidity, ventilation, etc.) have a significant effect on the response of insects at different stages. These conditions vary depending on height and proximity to walls, doors, ventilation, equipment, etc. (Barrett *et al.*, 2023). Therefore, we must distribute the experimental units evenly throughout the rooms so that all treatments are equally affected by this environmental variability, as these factors can create microclimatic conditions even within enclosed and supposedly controlled spaces.

Nevertheless, in certain instances, it is advised that the complete randomisation of the experimental units be limited. This is referred to as local control. Although most experimental units in insect studies comprise hundreds or thousands of insects (see the standard protocol by Deruytter *et al.* (2023), which reduces initial variability and typically includes individuals from multiple origins, it is recommended that local control be implemented at the outset of the experiment if prior information on the origin of the individuals is available. To illustrate, in a larval fattening test, the larvae may originate from eggs laid by specific breeders that have been housed under disparate conditions, or from different weeks of laying. Consequently, it is inadvisable to allocate all the eggs from a cohort of breeders from a given week of laying to a single treatment. It is preferable to distribute these eggs equitably among the various treatments to circumvent pre-experimental effects. Fasce *et al.* (2022) described how they perform local control to distribute mealworm trays from different laying weeks between treatments.

Recommendations for the experimental design in insect studies

In addition to considering the fundamental principles of experimentation outlined in the preceding section, when designing an experiment with insects, three further general recommendations can be made:

1. Using harmonised methods. Each of our experiments contributes a piece to a puzzle that we are all building together. For the pieces of this puzzle to fit together correctly, it is important that the results obtained by the different teams are comparable. In this way, we can more easily build the pillars of knowledge and make faster progress in each area. This type of standardised or harmonised methods have been developed for other zootechnical species for decades (e.g. in poultry and pigs (Bedford *et al.*, 2016), in rabbits (Fernández-Carmona *et al.*, 2010), and we already have some proposals for insects (e.g. for nutrition trials on black soldier fly (BSF, *Hermetia illucens*) larvae (Deruytter *et al.*, 2023). No protocol is perfect, but if we all follow the same procedure, our results will at least be comparable. In this sense, the main objective of the recent Cost Action CA23127 (https://www.cost.eu/actions/CA23127/) is to move in this direction.
2. Reduce sources of variability. When we perform a hypothesis test, we always relate the variability generated by the treatment to the unexplained variability (error). The greater the change caused by the treatment, or the smaller the error, the fewer the replications and the easier it is to obtain a sig-

nificant result (Morris, 2006). To reduce the error component, we must try to reduce the response variability associated with environmental effects, both at the beginning and throughout the experiment. In insect experiments are recommended to control three source of variability: (i) use the same source of animals to avoid genetic variations; (ii) try to keep the environmental rearing conditions and care routines as constant as possible throughout the experiment and ensure that all experimental units are exposed to the same conditions; (iii) it is important to ensure adequate health conditions and reduced mortality, as subclinical diseases increase the inter-individual variability (Howard, 2002).

(3) Correct characterisation of the treatment under study. In most of our experiments, we are usually evaluating a new raw material, genetic type, housing, management, pathogen, etc. For other researchers to be able to reproduce or compare our results, it is essential to carry out a perfect characterisation of our treatment in the material and methods section. For instance, Pascual *et al.* (2024), in evaluating six raw materials for use in feeding yellow mealworms, provide a complete description of the chemical composition and granulometry of the raw materials.

Different experimental designs in insect studies

A variety of experimental designs can be employed in animal studies, as evidenced by the classical publications (Kaps and Lamberson, 2017; Morris, 2006). The utilisation of mixed models for the analysis of data with repeated measures enables the adequate modelling of the data and the application of statistical tests that minimise bias, e.g. as described in Littell *et al.* (1998). Nevertheless, three principal designs are predominantly employed in the context of insect studies:

(1) Unchanging factorial designs with a single observation in time. This is exemplified by studies evaluating the impact of different feeds or rearing systems on the chemical composition of larvae. In such studies, different treatments may be applied at varying levels, with or without interactions. For example, Oonincx *et al.* (2015) compared different diets consisting of food by-products applied on insects of four different species. However, the outcome is a single response value obtained at post-sacrifice of the larvae. From a frequentist approach, after considering the recommendations of this section for the experimental design and the obtained data can be analysed using a generalised linear model (GLM).

(2) Repeated measures design. An illustration of this design is provided by the study of the growth of larvae over time, where data is collected from the same experimental unit (either the larva or the crate) at various points in time. For an ANOVA analysis to be deemed valid, the Huynh-Feldt condition must be met (Huynh and Feldt, 1970). This stipulates that equality of variance over time and pairs of measurements from the same experimental unit must be equally correlated over time. However, this is not typically observed in experiments with repeated measures involving living organisms. When the same treatment is applied over time to the same insects, the data from each experimental unit (insect, tray, etc.) may be more correlated with each other than data from different experimental units (this can be addressed with a split-plot design (Damon and Harvey, 1987). Additionally, the variance and covariance may change over time, which requires modelling. The application of ANOVA at each time point may result in the generation of erroneous standard errors, potentially leading to the formulation of inaccurate conclusions.

(3) Change-over designs. Let us consider a research scenario in which we wish to examine the impact of dietary fat content on the fat content of beetle eggs. In this instance, we could employ a change-over design, whereby some beetles are fed a fat diet and others a fat-free diet, and after a two-week period, the diets are reversed. This design is optimal for the elimination of the effect of the experimental unit, as all treatments are performed for each unit (balanced Latin square). This approach allows us to minimise the sample size; however, it is challenging to apply it to insects. Most insect stages are relatively brief, and these designs require an adaptation period, the absence of a permanent effect of the previous treatment, and short-term effects.

3 Rearing and production data analysis

Before, during and after any insect rearing or production experiment, one should have some data to analyse. In this part, we will focus on production parameters such as yield, survival, and growth. Normally, one should have already determined the statistical analysis method during the design of the experiment based on the hypothesis. The appropriate statistical method depends on several factors. For example, the measurement can either be obtained once during the trial, hereafter single measurement (e.g. harvest yield) or multiple times (e.g. tracking growth parameters), and the data can be qualitative or quantitative. The nature of the data will affect how they are analysed. It is important to note that in most cases, there are different correct ways to assess the same dataset. In the following sections, we will frequently refer to growth data as this is a very common parameter, but the suggestions are applicable to many other types of data (e.g. metabolic rates, survival rates over time, or substrate temperature). Finally, it is always advisable to contact a statistician when you are unsure how to handle the data or perform an analysis for the first time.

Single measurement analysis

Most data from experiments will fall within the 'single measurement' category, as this includes every parameter that is measured on a single occasion for each replicate. This may include but is not limited to traits such as the harvest yield, the maximum average weight of the larvae, survival/mortality, protein content of the larvae, feed conversion ratio, and so forth. It is important to note that each observation or measurement is independent of the others, which is the main difference with the repeated measure analysis that will be discussed subsequently.

As most of the time in statistics, this kind of data can be analysed using a variety of methods. However, in practice, either an ANOVA or a linear regression (LR) analysis is typically employed. From a theoretical standpoint, ANOVA is just a special case of LR, wherein the predictor variable assumes the form of a categorical variable comprising multiple levels. An ANOVA (one-way or two-way) is often used when working with a qualitative independent variable, such as when assessing different feedstocks based on fish, meat, fruit, and vegetables. Before an ANOVA can be used, two assumptions must be satisfied (besides independence). Firstly, normality or the assumption that the data is normally distributed in each group. This can be visually checked for using histograms and QQ-plots or can be formally tested using, for example, a Shapiro-Wilk test. Secondly, the assumption of homoscedasticity, or homogeneity of the variance, must be met. This means that the variance should be equal across the different groups. This can be visually inspected by scale-location plots or tested formally using Levene's test.

In the case of a continuous independent variable, the use of a LR is appropriate. For example, when an increasing protein content of the feed is assessed (e.g. 5, 10, 15, 20, 25%). In principle, protein concentration could be treated as a categorical variable with concentration levels as different factors and assessed using an ANOVA. Nevertheless, the LR method is to be preferred. The advantage of LR over ANOVA is that fewer model coefficients need to be estimated, which is particularly relevant when interaction terms are included in the model. Reducing the number of estimates increases statistical power. Furthermore, it allows researchers to interpolate values or calculate optima or minima, which can be used to make meaningful inferences about the experimental setup beyond the discrete data points collected. This may help to understand non-linear trends and improve comparability between treatments using limited data.

The same assumptions as for the ANOVA still apply, with the normality of the residuals and the equal variance of the residuals across the range of the independent variable. On top, the LR also assumes a linear relationship between the dependent variable (e.g. yield) and the independent variable (e.g. protein content).

In practice, it is not uncommon for the underlying assumptions to be violated, which renders the use of ANOVA or LR inappropriate. This does not mean, in most cases, that the data cannot be analysed; rather, it suggests that the results should be adjusted to account for these violations. Some of the more frequently encountered violations of the assumptions are as follows: violations against the homoscedasticity assumption when assessing the average weight of the larvae (e.g. in different feedstuffs) as it is to be expected that the variability for small larvae is much lower than for large larvae (in absolute values). In such instances, the data may be transformed (e.g. log-transformation), meaningful weights may be set to stabilise residual variance or alternatively, GLMs may be employed with an alternative family or link function (e.g. Poisson, see below). In the event of a violation of the independence assumption, it is possible to resolve this by undertaking a multilevel analysis (see below). Finally, when the normality assumption is violated, data transformation can be considered to meet the assumption. Depending on the nature of the data, commonly used methods for transformation include logarithmic, square root, or Box-Cox transformation. If transformation does not resolve this issue, it is possible to employ non-parametric alternatives such as the Kruskal-Wallis test in the context of ANOVA.

Violations against independent measurements

This section aims to introduce mixed-effect modelling, the conditions wherein these should be employed and how this can be used to construct treatment dependent insect growth curves and how to incorporate interactions. When performing an unpaired *t*-test, or an LR or ANOVA, it is essential to assume that all measurements are independent of one another and that the subset of the population under examination is representative of the entire population at the specific point in time. This is also referred to as cross-sectional data.

However, this assumption is invalidated when measurements are not independent of one another, resulting in autocorrelation of the data. This phenomenon occurs in the context of hierarchically structured data, wherein observations are made at multiple levels, ordered according to a hierarchical structure. To illustrate, consider a feed experiment with crickets where the weight of individuals is determined at harvest. Due to the experimental design, the mother of each individual is known. In this context, it is expected that the performance of siblings of a given mother will be more similar compared to the performance of offspring of the other mothers due to the influence of shared genetics. In this instance, the mother may be regarded as a distinct level in the hierarchal structure. There is not necessarily a specific interest in this higher level, but not accounting for its effect may result in correlations between measured values being obscured due to the variance that the higher level imposes. Multilevel modelling with fixed and random effects, also referred to as mixed-effects modelling, is one way to incorporate these variances into the model. If the mother is regarded as a level, the mother would be incorporated into the mixed-effect model as a random effect, whereas the tested treatment would be designated as the fixed effect.

Mixed-effect modelling is particularly useful when analysing growth data for insects. When assessing insect growth, it is common practice to measure the same group of insects at time points from the start to the end of the experiment. All measurements taken on the same group of insects are correlated with prior or subsequent measurements on the same group. This constitutes an example of longitudinal data (a case of hierarchical data), which must be analysed accordingly, as is discussed in the next paragraph. The issue of measurements taken on the same group of insects being correlated with prior or subsequent measurements of the same group can be avoided by measuring the insects only once and removing it from the study after measurement. This would eliminate the sampling effects and ensure the growth is assessed consistently over time rather than being influenced between differences between individual insects. However, this would considerably expand the scope of the experimental design with each additional intermediate measurement.

When constructing a growth model using mixed-effect modelling, the initial weight (intercept) is specific to each replicate, as is the slope followed by each replicate. If a specific replicate exhibits a greater initial weight due to chance, there is a heightened probability that it will demonstrate superior performance in subsequent weeks. Additionally, if a single replicate exhibits superior growth relative to the others, the slope of this replicate will also be greater. These factors may be included as random effects within the model. The fixed effects may include, for example, the treatment as well as the age of the insects, with an interaction term between both. Should the interaction term yield significant differences between the levels of the treatments, this would indicate that the growth rate differs significantly between treatments. A similar approach can be employed when assessing mortality rates, etc. It should be noted that all standard assumptions for linear regression, namely a linear relation between variables, normality and homoscedasticity of the residuals, also apply to mixed effect modelling.

In conjunction with the main effects of the investigated factors, significant interaction terms provide biologically highly relevant information yet need to be interpreted with caution and particularly require attention regarding post-hoc contrasting. For instance, when the factors diet and strain-specific genetic background reveals a significant interaction term for a given trait tested in a fully crossed factorial design, pairwise statistical contrasts between levels within one factor across levels of another would be formally inappropriate. Accordingly, the effect of different strain genetics must be tested only within individual diets, or vice versa, as detailed in Sandrock *et al.* (2022).

Violations against normality and equal variance

Violations against the normal distribution of the residuals and the equal variance of the residuals are frequently encountered in insect research. To illustrate, consider the case of growth data. In general, when larvae are small, the variability in weight is minimal (e.g. average 5 mg, standard deviation 1 mg) and for larger larvae it can be considerable (e.g. average 100 mg, standard deviation 20 mg) in absolute terms. This constitutes a clear violation of the equal variance assumption, thereby rendering a normal LR impossible. In many cases, these issues can be addressed by using GLM, which allows for the distribution of the variance to deviate from normality. Data transformations are also frequently used to obtain similar results but may not be preferred. In particular, log-transformations should be avoided when the data contains zeros (O'Hara and Kotze, 2010).

When dealing with discrete data or right-skewed continuous data, respectively, the Poisson distribution or Gamma distribution can be used with a logit link function. There are several advantages to utilising this distribution instead of the normal distribution. Firstly, in linear regression, the normal distribution allows for predictions to be negative, which is not always reflective of reality in experimental settings (e.g. survival, size, length, number of eggs). Both the Poisson and the Gamma distributions are defined in the interval $[0, +\infty)$. Using the logit link is analogous to a logarithmic transformation, obviating the necessity for data back-transform due to the presence of zeroes (St-Pierre *et al.*, 2018). Several other distributions can be useful in such cases, particularly when there are more zeros than expected. This is exemplified by survival studies, where only a small proportion of individuals are expected to die. In such instances, the negative Binomial or the zero-inflated Poisson regression models may be used. We do recommend reaching out to an expert when dealing with this kind of data.

Binary data, also known as dichotomous data, is defined as data that has only two potential values, 0 or 1. Although it is less common in insect research than continuous data, such data may be encountered with greater frequency than may be anticipated. To illustrate, a mating/egg-laying event may occur or not, a single insect may be either dead or alive, an insect may choose to turn left or right in a Y-tube, a certain pest species may be present or not, and so forth. In such instances, the use of multiple logistic regression is an appropriate methodology for the analysis of the data, with the objective of establishing associations and formulating predictions. This can be achieved through the implementation of a GLM, using the binomial distribution as the underlying statistical framework. Keep in mind that this method still assumes independence. This is not the case, for example, if the same female mates on more than one occasion. The Wald's test or likelihood ratio test may be used to ascertain whether the independent variable exerts a significant influence on the dependent variable.

A combination of GLM and multilevel models can be used to create a generalised linear mixed model (GLMM), as is done for growth data. A good example is the analysis conducted by researchers from University of Turin (Biasato *et al.*, 2024). Similarly, accounting for overdispersion, which is when the variance of the response is greater than what the model assumes, needs to be accounted for by either adjusting dispersion parameters using models of the quasi family, or introduce data-specific observation-level random effects by extending a GLM to a GLMM (Sandrock *et al.*, 2024).

Violations against linearity

This section, obviously, only applies to generalised linear regressions. Nevertheless, it is important to acknowledge that in numerous instances, the relationship between the dependent and independent variables does not adhere to the strict linearity assumed by the model ($y = a + b * x$). Attempting to force this model upon such cases may result in erroneous conclusions. In many cases, the relationship between two variables is concave, convex, exponential, or otherwise non-linear. It should be noted that this would be discernible as a still-existing trend in the residuals of a fitted linear model. In this case, it is feasible to incorporate polynomial terms of a specific degree ($y = a_0 + a_1x + \cdots + a_{n-1}x^{n-1} + a_nx^n$). As the complexity of the polynomial increases, the model will demonstrate a superior fit to the data. However, it also increases the risk of overfitting. In general, a lower-degree polynomial is more robust against outlier values. This methodology can be employed to ascertain the optimal conditions for insect performance when subjected to varying temperatures, protein concentrations, and other environmental factors. It can also be used to enhance the precision of growth curves. Some more advanced (non-linear) models may be used, as the relationship between time and the size of the larvae frequently resembles a sigmoid function (Laganaro *et al.*, 2021; Sripontan *et al.*, 2020). The aforementioned models are not currently implemented on a regular basis, and their use depends on the taking of frequent intermediate measurements. Nevertheless, they may offer a more precise model and may become more prevalent in the future.

Model selection

The selection of the optimal model represents a crucial step in the statistical analysis, especially in the case of intricate models comprising multiple variables, interactions and random variables. As always, there are several methods that may be employed to perform the selection. One way to select is based on the p-values of the various independent variables or interactions. An alternative approach is to select a more general model based on the Akaike Information Criterion (AIC) or Bayesian Information Criterion (BIC). BIC generally results in a simpler model. AIC and BIC are better suited when the goal is to build a predictive model, whereas the p-value selection is more suitable when the aim is to identify which parameters are specifically responsible for a given phenomenon.

The present study has focused on proposing tools and guidelines for data analysis in insect studies using frequentist methodologies, given their extensive use. However, there is an alternative that is gaining more and more popularity, the Bayesian statistics. Bayesian methodology allows a more explicit approach to probability through iterative processes, and its main advantage is that it allows a more faithful and direct interpretation of the results. As the utilisation of Bayesian techniques in the field of insect studies continues to evolve, the development of comprehensive guides or recommendations for their application may be advantageous.

No matter which method is used, there are two principal approaches (and mixes thereof) to identifying the optimal model: forward or backward selection. In forward selection, the null model (comprising only the intercept) is initially employed. Subsequently, the impact of each independent variable is evaluated in sequence, with the variable exhibiting the most favourable outcome in terms of AIC, BIC or p-value being incorporated into the model. This process is repeated until the specified criteria (e.g. a p-value $\leq$ 0.05) are no longer met by any variable. In contrast, backward selection starts with the saturated model (i.e. the model comprising all variables and interactions) and variables that fail to meet the specified criteria are excluded sequentially. Backward selection is often used and preferred when the number of parameters is relatively limited compared to the number of observations. If this is not the case, the estimations of the saturated model estimations may not accurately reflect the truth, and the incorrect parameters may be removed. It is possible to derive multiple valid models for the same data set using a different set of independent variables. This can, for example, occur when multiple independent variables are highly correlated with one another. This is evident when assessing both the length and weight of mealworm larvae on the reproductive output of the beetle. Given the high degree of correlation between length and weight, it is inadvisable to include both in the same model. In such instances, the optimal model can be identified through the assessment of its AIC or BIC, with a smaller value indicating a superior fit).

4 Microbiological and toxicological studies

Mass-reared insects are exposed to a plethora of microbial influences from their environment and rearing substrates and microbial load can have significant impact on insect health, product safety, and quality (Van Campenhout and Eilenberg, 2021). Equally important are toxicological concerns, such as the potential accumulation of harmful substances like heavy metals, pesticides, or mycotoxins from the rearing substrates, which can pose serious risks to both insect health and the safety of derived products (Heuel *et al.*, 2023). Thus, it is crucial to systematically assess and monitor these potentially harmful factors in controlled, artificial settings. This process should ideally follow established and standardised protocols such as those published by the International Organisation for Standardisation (ISO), to ensure consistency, reproducibility, and comparability in the evaluation and management of risk factors.

Microbiological studies

Established techniques such as plate counting, disc diffusion assays, and next-generation sequencing are employed for the quantification of microbial abundance, testing their antimicrobial resistance, and characterising microbial communities, respectively (Wynants *et al.*, 2019). Microbiological studies on insects used for food and feed focus on microbial abundances, community composition, and the presence of antibiotic-resistant bacteria to ensure food safety and assess potential health risks (Garofalo *et al.*, 2017). Investigating antibiotic resistance is crucial not only to prevent the spread of resistant pathogens through insect-based foods but also to explore the potential of insect-derived antimicrobial compounds for pharmaceutical and biotechnological applications (Manniello *et al.*, 2021; Osimani *et al.*, 2018). Both abundance and composition of microbiota can strongly vary based on the experimental and production framework (Gorrens *et al.*, 2022). Albeit certain mass-reared insects such as BSF larvae have been shown to have antimicrobial effects on certain pathogenic bacteria including *Salmonella* sp. (Lalander *et al.*, 2015) or *Escherichia coli* (Erickson *et al.*, 2004), contamination with harmful microorganisms is a relevant risk (De Smet *et al.*, 2021).

Plate counting involves diluting the samples, spreading them on suitable agar plates (containing non-selective, selective, or differential media), and counting the colonies grown after incubation under controlled conditions for a defined time. The objective of total bioburden assessments is to quantify the overall microbial load, whereas pathogen detection employs selective media and/or conditions to promote the growth of specific target microorganisms (Chauhan and Jindal, 2020). To distinguish between two or more species that are difficult to identify, differential media can be used to test certain biochemical processes.

The obtained microbial counts are expressed as colony forming units (CFUs) per unit of sample (e.g. CFU/g or CFU/ml), with the final count adjusted to the sample's dilution level. As both quantification and detection need to be conducted in multiple replicates (typically 3-5 biological replicates) to ensure reliable data, CFU counts are typically summarised as mean ± standard deviation. These data are often highly skewed and differences in CFU counts can span magnitudes, thus, log-transformation is often appropriate to enhance comparability and enable subsequent statistical testing. Comparative analyses include simple *t*-tests or analyses of variance, or non-parametric tests such as Kruskal-Wallis to compare counts across different groups (Ilstrup, 1990). For pathogen detection, the presence absence (binary data) of specific species may often be sufficient, but the detailed interpretation of these data is coupled to regulatory thresholds introduced by national authorities. In addition to quantification and detection of specific microorganisms, traditional cultivation techniques can be enhanced by sequencing techniques to taxonomically and metabolically characterise (novel) species (Bonnet *et al.*, 2020; Gorrens *et al.*, 2021).

Disk diffusion assays are predominantly used to test the antimicrobial susceptibility of isolated microbial strains against antimicrobial agents. Paper discs loaded with antibiotics are placed in the centre of agar plates inoculated with the microbial isolate to be tested (Bonev *et al.*, 2008). After diffusion into the agar, the agent may inhibit microbial growth around the disc by inducing a clear inhibition zone. The data from these tests consist of inhibition zone diameter measurements, which are compared to standardised charts to determine whether an isolate is susceptible, intermediate, or resistant to the tested agent (Clinical and Laboratory Standards Institute (CLSI), 2020). This method is particularly useful for evaluating novel probiotic candidates by assessing their potential resistance to common antibiotics, which would render them unsuitable for probiotic applications (Liu *et al.*, 2023; Suraporn *et al.*, 2024). In the context of insect mass production, this method can be applied to monitor and manage microbial communities associated with insect rearing systems. For instance, testing the susceptibility of bacteria from insect gut microbiota or rearing substrates can help identify effective antimicrobial agents to control harmful pathogens, ensuring the health and productivity of insect populations.

Microbial communities, both of insects and the substrate, play a central role in insect health and development and can affect product safety. The gut microbiota supports digestive, immune, and detoxification processes in the insect's physiology (Bruno *et al.*, 2019). Its composition is strongly shaped by the provided feed and rearing conditions, and methods such as 16S rRNA gene sequencing is frequently used to monitor microbiome composition and functions (Klammsteiner *et al.*, 2021). Analysing the microbiome using sequencing approaches (e.g. amplicon or shotgun metagenomic sequencing) generates vast amounts of data that require adequate data (pre)processing, statistical validation, and interpretation. The preprocessing of microbiome data can be summarised in three main steps: filtering (samples and features, chimera removal), data normalisation, and sequence annotation (clustering of operational taxonomic units or amplicon sequencing variants, taxonomic assignment). The resulting feature tables, containing data on microbial (relative) abundances, are used to calculate alpha diversity (describing the diversity within a sample) and beta diversity (describing the diversity between samples). These diversity measures can then be statistically tested using multivariate statistics (e.g. principal coordinates analysis, non-metric multidimensional scaling, permutational analysis of variance), differential abundance testing (e.g. linear discriminant analysis of effect size), or network analysis (Liu *et al.*, 2021). This topic is further discussed in more detail in BugBook chapter dealing with insect microbiome (Auger *et al.*, 2025). Moreover, the rise of machine learning and artificial intelligence has led to novel approaches for enhancing the informative value of microbiome data (D'Elia *et al.*, 2023; Marcos-Zambrano *et al.*, 2023). These advancements may enable the prediction of microbiome responses to the changing environmental factors (e.g. substrates, rearing conditions) and facilitate deducing information on health and disease, such as microbiome imbalances or dysbiosis, before they manifest in reduced rearing performance or product quality. Although we are far from efficiently exploiting and optimising the potential of the insect's gut microbiota, it offers a promising opportunity to sustain insect health and in turn reduce the risk of pathogens or toxin-producing microorganisms.

Toxicological studies

The safety of insect-based products also relies on toxicological assessments. These analyses target potentially harmful substances including heavy metals, pesticides and their residues, microplastics, and mycotoxins, which could end up in the insect biomass from the fed substrate or in the resulting frass (Alagappan *et al.*, 2024; Traynor *et al.*, 2024). Toxicological characterisation is supported by both chemical analyses and bioassays, and while insects such as BSF are generally deemed safe, deviations in rearing conditions could lead to the accumulation of harmful substances. Recent research has shown, that microplastics are efficiently excreted by BSF and do not accumulate in biomass (Heussler *et al.*, 2024; Lievens *et al.*, 2023). However, heavy metals such as arsenic, lead, cadmium, mercury can accumulate in the biomass – albeit the concentrations comply with legal limits imposed by the EU (Grosso *et al.*, 2024; Purschke *et al.*, 2017). Effects of mycotoxins and pesticides are dependent on type and dosage and research in this field is scarce. Purschke *et al.* (2017) reported that substrates spiked with mycotoxins (aflatoxin B1, aflatoxine B2, aflatoxine G2, ochratoxin A, and zeralenone) and pesticides (chlorpyrifos, chlorpyrifos methyl, and pirimiphos methyl) at common concentrations had no adverse effects on larval development, nor did these contaminants accumulate in the insect biomass, which is important for product safety reasons (Heuel *et al.*, 2023). But pesticides such as deltamethrin were shown to be highly toxic to BSF larvae even well below the legal concentration thresholds and synergistic reactions with other compounds such as piperonyl butoxide are likely (Meijer *et al.*, 2025).

Analytical methods to investigate these contaminants include inductively coupled plasma mass spectrometry (ICP-MS) for heavy metals (Grosso *et al.*, 2024; Purschke *et al.*, 2017) and liquid chromatography often coupled with mass spectrometry for e.g. pesticides (Meijer *et al.*, 2025). The measured concentrations are reported as e.g. mg/kg of sample or in parts per million (ppm) or parts per billion (ppb) and are normalised for dilution factors. Data generated by these methods are summarised using descriptive statistics (measures of centre and variation) and common comparative tests such as t-tests or analysis of variance can be applied for group comparisons and hypothesis testing. If data are not normally distributed, appropriate transformation methods (e.g. log, square root, boxcox, or cubic transformation) should be applied prior to testing, however, interpretation of the data may be hampered due to distortion from the transformation. In addition, correlation or multivariate can be conducted to test whether specific contaminations follow similar or opposing trends. Analogous to the interpretation of pathogen CFU counts, thresholds for heavy metal and toxin concentrations follow legal requirements set by the responsible authorities (e.g. European Food Safety Agency, Food and Drug Administration, or other national bodies).

5 Perspective methods of behavioural modelling for black soldier fly

Reproductive behaviour

Reproductive behaviour is complex. Take for example, a breeding population of BSF of which there may be some individuals that do not mate at all, some that only mate once, and some of that mate multiple times (Chiabotto *et al.*, 2024; Hoffmann, 2021; Jones and Tomberlin, 2021; Muraro *et al.*, 2024). The matings that an individual female receives from one or multiple partners may either be too few, adequate, or more than enough needed to completely fertilise her eggs (Dickerson *et al.*, 2024). Some females may not lay eggs at all (Dickerson *et al.*, 2024; Lemke *et al.*, 2024b), while others lay the totality of their eggs in a single attempt, and still others take multiple attempts to lay their eggs over the course of their lives (Chiabotto *et al.*, 2024; Nakamura *et al.*, 2016). Of the eggs that are laid, these can vary in fertility and viability as well (Dickerson *et al.*, 2024; Nakamura *et al.*, 2016). This effectively means that the total reproductive potential of each female is a theoretical quantity only, since in addition to the forestated factors, eggs can also be laid outside of traps (Dickerson *et al.*, 2024; Harjoko *et al.*, 2023; Lemke *et al.*, 2024a) or reabsorbed by the female (Lemke *et al.*, 2024a).

Statistical methods need to be developed for insect production to account for the nuanced complexity of behavioural outcomes, including mating success, fertilisation rates, egg viability, and overall reproductive potential. Given that behavioural count data are typically highly skewed and zero-inflated, such methods include (a) non-parametric statistics (b) truncating zeroes from the data set, or (c) using a class of models that can handle excess zeros, such as Hurdle models (Mullahy, 1986, 1997). While this section focuses on Hurdle models, it is important to know that they cannot be unilaterally applied to situations with excess zeros, since in some cases Poisson or negative binomial models will perform better (e.g. based on Akaike's Information Criterion) (Green, 2021; Hofstetter *et al.*, 2016; Warton, 2005).

Non-parametric statistical analysis

Although an axiom states 'all biological traits are normally distributed', this is simply not true. Non-parametric methods are necessary when sampled data is not normally distributed, which can be caused by several underlying factors (Kraska-Miller, 2013). These include the presence of outliers, skewness, low sample size, mixed distributions, among others. Because count data is both discrete (i.e. non-continuous) and truncated at zero (viz., there cannot be negative counts), a low mean will almost certainly entail a non-normal distribution. In addition, whenever the standard deviation is greater than the mean, this effectively produces a nonintuitive negative value for metrics bounded at zero (Jones and Tomberlin, 2021). As such, summary statistics reported by future research should report median values, as an alternative or addition to reporting means (Lemke *et al.*, 2024a), since unlike means, medians are not influenced by extremes or skewed data. Non-normality can be confirmed by plotting residuals or confirmed statically by the Shapiro-Wilk or Kolmogorov-Smirnov tests, while unequal variance (heteroskedasticity) can be seen when plotting residuals versus fitted values (fits) or by conducting the Bruesch-Pagan test (Kraska-Miller, 2013).

Of course, when data does not fit the parametric assumptions then it might be appropriate to transform the raw data to approximate a normal distribution (such as through the square root, reciprocal, or log functions). However, since this distorts the data, it often makes interpretation of biological meaning much more tenuous, since the scale is not the same as that which the data were recorded (Osborne, 2002). Although transforming data and performing parametric analysis would be preferred if calculating the differences in means is critical, as research questions become more complex, non-parametric statistics (as discussed in previous sections). However, sometimes data can be so heavily zero-inflated that fitting a GLM with a non-Gaussian distribution (Poisson, Negative Binomial, or Gamma with log-link) to the data still fails. In this case, what is required is to either (a) truncate the zeroes, or (b) use a specific class of model that can handle the excess zeroes, such as Hurdle models (Mullahy, 1986).

For statistical analysis of non-normal data, tests are needed which do not rely on the mean value. Non-parametric methods are capable of handling more complicated data structures such as those involving repeated-measures, time-series, multiple comparisons, and non-linear relationships (Kraska-Miller, 2013). For analysis of categorical variables, common methods include Chi-square test, Mann-Whitney U, and Kruskal-Wallis H test (often with Dunn's post-hoc for multiple comparisons, or a Conniver-Iman test); while Kernel regression can be used to analyse time-series data. For continuous variables common methods include local regression (LEOSS), permutational models including permutational analysis of variables (PERMANOVA) and permutational analysis of covariance (PERMANCOVA) and general additive models (GAMs). For a more thorough discussion of each of these, we direct the interested reader to standard texts on multivariate analysis, e.g. Legendre and Legendre (2012). Instead, the remainder of this section will focus on the application of hurdle models, their advantages, and use for modelling behavioural count data.

Applications and extensions of Hurdle models

Count data mating (and similar metrics) often has excess zeroes because of underlying ecological factors. For instance, for BSF mating frequency, most mating typically occurs during the early-to-mid morning and the first few days of being in cages (Dickerson *et al.*, 2024). Because of this, there are many times during a 6- or 7-day experiment or breeding cycle in which zero mating would be observed for the study population (assuming benchtop-scale and no additional flies were added to the cage) (Dickerson *et al.*, 2024; Lemke *et al.*, 2024b, 2024a). Additionally, optimal mating is also predicated on the presence of ultraviolet light (Zhang *et al.*, 2010) interfacing with a wide range of demographic (including body size, age, physiology, condition, previous mating status/experience) and environment factors (including, but not limited to temperature, humidity, barometric pressure, cloud cover) (Meneguz *et al.*, 2023). When these conditions vary, so too do the spread, range, extremes, and total amount of mating counts. The same too can be applied to other count data including oviposition frequency and counts of fertile eggs. As such, hurdle models can be applied to study a wide range of behavioural data and should be used to gain a more nuanced understanding from heterogenous behavioural count data, especially at benchtop scale.

6 Data methods in consumer research

Consumer research is an interdisciplinary field that investigates the behaviours, preferences, and decision-making processes of consumers. The methodologies employed in this domain are diverse, encompassing qualitative, quantitative, and mixed-methods approaches. Each methodology offers unique insights into consumer behaviour, enabling researchers to develop comprehensive understandings that inform marketing strategies and policymaking. This chapter explores the various data methods utilised in consumer sciences, with a particular focus on the contributions that have significantly advanced the understanding of consumer behaviour in the context of food systems and novel foods like insects. Conducting consumer research for insects as food and feed is very important in increasing the consumer acceptance of insects by examining the role of affective (versus cognitive) communication messages about insect-based products (Onwezen *et al.*, 2022).

Qualitative methods in consumer research

Qualitative research methods are essential in consumer sciences, as they provide rich, contextual insights into consumer motivations and behaviours. Techniques such as interviews, focus groups, and ethnographic studies allow researchers to gather detailed narratives that elucidate the complexities of consumer decision-making (Zaltman, 2003). For instance, qualitative studies can reveal the underlying values and beliefs that drive consumer choices, particularly in the context of food systems and sustainability (Miah *et al.*, 2022), and in research dealing with insects for food (Mandolesi *et al.*, 2022). For example, qualitative methods like initial interviews and focus groups can be a starting point in the studies defining the strategies for the promotion of insect-based foods (Sogari *et al.*, 2017) or identifying the potential perception of consumers, when little or no information is available (Spartano and Grasso, 2021). Moreover, qualitative methods facilitate the exploration of emergent trends and consumer sentiments. Banister and Booth emphasise that qualitative research can develop hypotheses that quantitative methods later test, thereby enriching the overall research process (Banister and Booth, 2005). This iterative approach enhances the rigor of qualitative studies, allowing researchers to refine their questions and methodologies based on emerging insights. However, qualitative research is not without its challenges. The subjective nature of qualitative data can lead to biases in interpretation, necessitating rigorous methodologies to ensure credibility (Zanoli and Naspetti, 2004). Techniques such as member checking and triangulation can help mitigate these biases, enhancing the validity of qualitative findings (Gummesson, 2005). Additionally, integrating consumer involvement in qualitative research can further enrich the data collected and provide deeper insights into consumer preferences (Thach *et al.*, 2021).

Quantitative methods in consumer research

Quantitative methods play a crucial role in consumer sciences, providing statistical rigor and the ability to generalise findings across larger populations. Surveys and experiments are common quantitative approaches that enable researchers to collect numerical data on consumer preferences, purchasing behaviours, and demographic information. For example, studies examining consumer preferences for organic food often utilise structured questionnaires to gather data from significant sample sizes, allowing for robust statistical analyses (Banister and Booth, 2005). Statistical techniques such as regression analysis, factor analysis, and structural equation modelling (SEM) are frequently employed to analyse relationships between various consumer variables (Berger and Wyss, 2020; Scott and Uncles, 2018; Vartiainen *et al.*, 2020). These methods not only quantify consumer behaviour but also facilitate hypothesis testing regarding the impact of marketing mix elements on purchasing decisions (Belk and Kozinets, 2005). The ability to draw correlations and causal inferences from quantitative data is invaluable for businesses seeking to optimise their marketing strategies and enhance consumer engagement (Scott and Uncles, 2018). Despite their strengths, quantitative methods face challenges, particularly in ensuring that samples are representative of target populations. Researchers must be vigilant in selecting appropriate statistical techniques and ensuring that the assumptions underlying these methods are met (Belk and Kozinets, 2005). Additionally, the reliance on numerical data may overlook the nuanced motivations and emotions that drive consumer behaviour, underscoring the importance of integrating qualitative insights (Zanoli and Naspetti, 2004).

Mixed-method approaches

The integration of qualitative and quantitative methods, known as mixed-methods research, has gained prominence in consumer sciences due to its ability to provide a more comprehensive understanding of complex consumer phenomena (Goulding, 1999, 2005). Mixed-methods research combines the strengths of both qualitative and quantitative approaches, allowing for triangulation of data and a more nuanced analysis of consumer behaviour (Thach *et al.*, 2021). For instance, researchers can employ qualitative discussions to explore consumer attitudes while simultaneously conducting quantitative surveys to evaluate purchasing behaviour (Goulding, 2005). The design of mixed-methods studies requires careful consideration of the relative emphasis placed on qualitative and quantitative components. Observational studies may also be considered mixed method, since they use a mixture of qualitative and qualitative techniques to observe consumer behaviour, and to interpret it (Naspetti and Zanoli, 2014). Researchers must ensure that the integration of methods is purposeful and that both components contribute meaningfully to the research objectives. This approach not only enriches the data collected but also enhances the validity of the findings by providing multiple perspectives on the research question (Goulding, 2005). However, mixed-methods research, like Q Methodology (Brown, 1980; Mandolesi *et al.*, 2022; Stephenson, 1953) presents unique challenges, particularly in terms of integrating findings from different methodologies. Researchers must develop clear frameworks for data integration and analysis to ensure that the combined results provide meaningful insights (Kleih *et al.*, 2022). The complexity of mixed-method designs necessitates a high level of methodological expertise and careful planning to achieve coherent and actionable conclusions (Goulding, 2005).

In the context of edible insect research, qualitative methods are particularly useful for exploring consumer attitudes, cultural perceptions, and barriers to acceptance through in-depth interviews and focus groups (Verbeke, 2015). Conversely, quantitative approaches, such as surveys and experimental studies, are effective for measuring consumer willingness to pay for insect-based foods, nutritional knowledge, and the impact of marketing interventions (Tan *et al.*, 2015). A mixed-method approach is beneficial when studying the relationship between consumer perceptions and actual purchasing behaviour, for instance, by combining qualitative focus group discussions with quantitative sales data analysis to assess market potential for insect-based products (Mancini *et al.*, 2019). This way, qualitative approaches highlight on the reasoning for the decisions and quantitative cover the aspects for the check-prove of the actuality of such decisions.

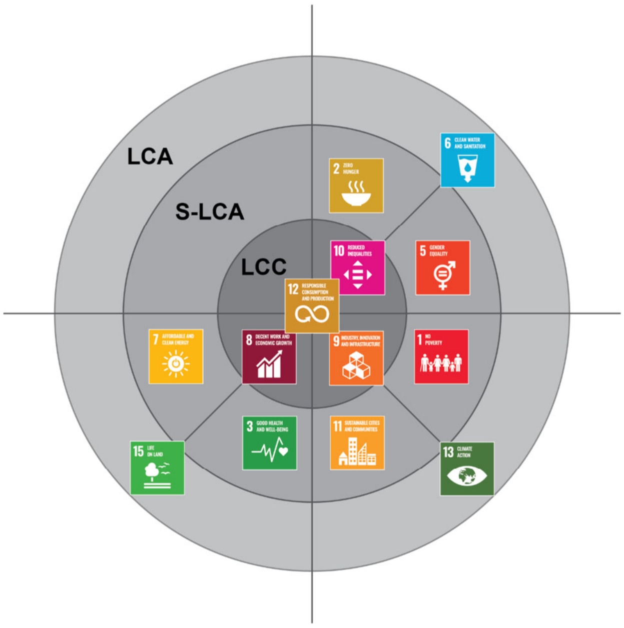

FIGURE 1 Scheme of potential integration of insect studies on LCA, S-LCA and LCC with the Sustainable Development Goals of United Nations.

7 Sustainability studies (Life Cycle Assessment, Life Cycle Costing, Social LCA)

Sustainability studies have become crucial in today's business world, integrating economic, environmental, and social dimensions into firms (Chang *et al.*, 2017). As concerns over resource limitations and environmental degradation grew, the concept of Life Cycle Assessment (LCA) took shape in the 1960s. Early developments in LCA emerged from the U.S. and Northern Europe, particularly following packaging studies. Initially, these efforts primarily focused on energy consumption and a few impact categories (Bjørn *et al.*, 2018). The International Organization for Standardization (ISO) since 1994, has been engaged in standardising requirements for conducting LCAs. Practitioners should note that the ISO standards of 14000 series outline the framework for LCA, setting a few important steps developing widely used guidelines (Finkbeiner *et al.*, 2006; ISO 14040, 2006; ISO 14044, 2006; Traverso and Valdivia, 2024). Since then, LCA-based approaches became the forefront of the holistic sustainability assessment, not only applicable for the insect studies, but important for their further development in the scope of Sustainable Development Goals (Figure 1).

LCA assessments can inform design changes, improving product sustainability (Pryshlakivsky and Searcy, 2013). LCA has a wide range of tools applicable for the assessment, such as impact assessment methods, e.g. IMPACT 2002+, ReCiPe (Goedkoop *et al.*, 2013), USEtox model (Hauschild *et al.*, 2008), ILCD 2011 (Wolf *et al.*, 2010), TRACI 2.1 (Ryberg *et al.*, 2014), and IMPACT World+ (Bulle *et al.*, 2019) the Environmental Footprint (EF) 3.1 (Andreasi Bassi *et al.*, 2023). More important

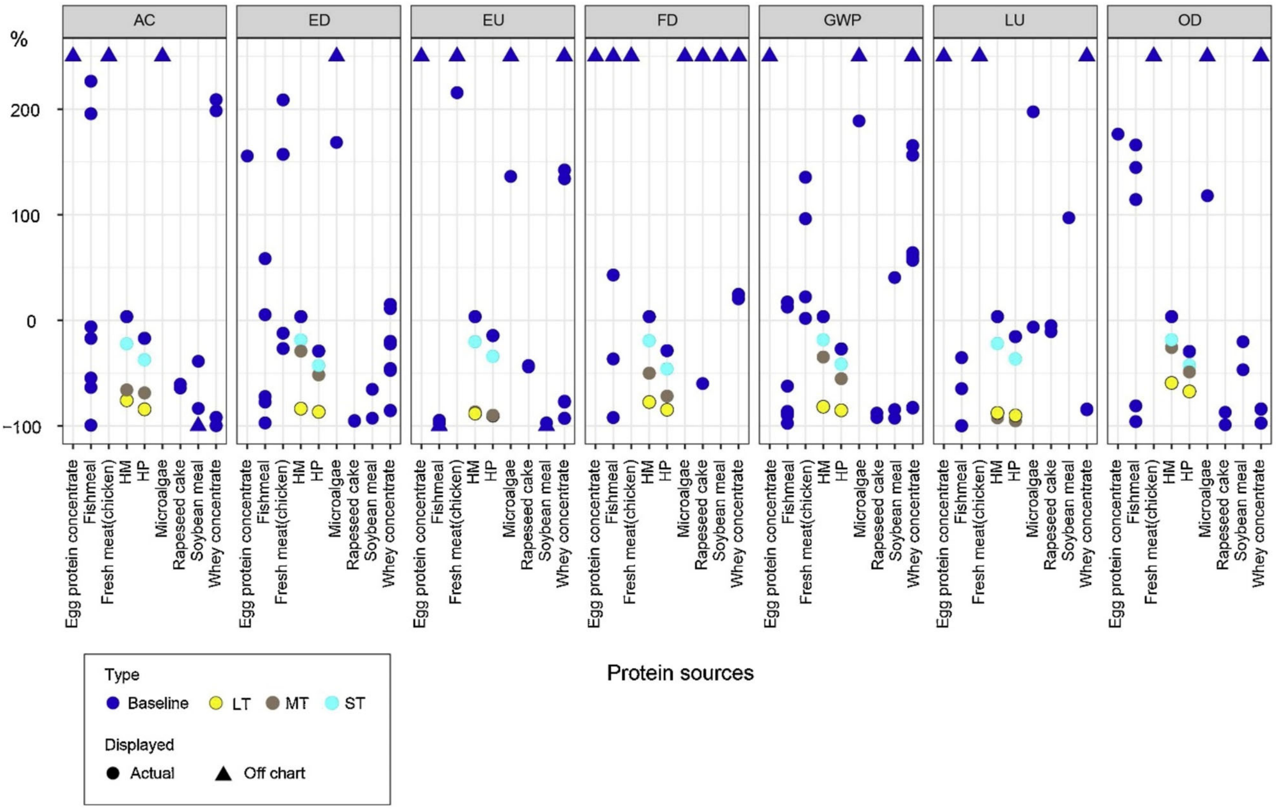

FIGURE 2 Environmental impacts of different sources of proteins (dry matter basis) weighted against HM, GWP, global warming potential; OD, ozone depletion; AC, acidification; EU, eutrophication; ED, energy demand; FD, freshwater depletion; LU, land use. Relative impacts are censored at −100% and 250% to maintain the readability of the plot and as triangles at these limiting values (Smetana *et al.*, 2019).

for the advancement of LCA in research is the development and maintenance of background and food-feed relevant databases: ecoinvent (Wernet *et al.*, 2016), Agri-Footprint (van Paassen *et al.*, 2019), Agribalyse (Colomb *et al.*, 2015), etc., which can be used to improve the speed and system character of the studies.

Availability of a wide range of methods and databases positively affected the progress of insect LCA studies (Halloran *et al.*, 2017; Modahl and Brekke, 2022; Oonincx and de Boer, 2012; Smetana *et al.*, 2016, 2019; Suckling *et al.*, 2020). However, it caused the application of very different assessment methods and background datasets from different databases in the studies dealing with different insect species, making them hardly comparable and data hardly interoperable. Thus, different studies considered various functional units (based on mass of the final product or amount of feed transformed, protein content or calorific value of yield biomass), approaches to the assessment (attributional to determine the environmental hotspots or consequential to define the potential effects on the market), system boundaries (cradle-to-gate, gate-to-gate, cradle-to-grave) (Smetana *et al.*, 2021). Despite the hurdles, a few studies compare the outcomes of LCA studies for different insects, traditional and alternative proteins sources. For example, Smetana *et al.* (2019) conducted a life cycle assessment (LCA) to compare the environmental impacts of different insect-derived products from the same production system with those of conventional protein sources (Figure 2).

In many cases the data from the studies is not open available, which limits the potential to reproduce the assessments. However, further developments are ongoing, and it is expected that the unifying methods for the LCA in insect research will be applied soon. It is possible to provide certain recommendations, considering the previous studies done in the field. First of all, it is necessary to follow the recommendations being established as a standard, for example PEFCR (Product Environmental Footprint Category Rules) and EF (Environmental Footprint) methods (Damiani *et al.*, 2022). Even though no specific PEFCR and EF exist for insect industry, general guidelines can be applied and followed (cradle-to-grave system boundaries, EF assessment method, primary data as much as possible, system expansion first). Functional units should represent the main function of the product or a system, and if assessment is completed for the mass unit – dry matter units should be considered. More than one functional unit is to be applied in each study (for example, mass unit in dry matter representation and protein content for meals).

Sustainability assessment is not complete without the assessment of economic costs (direct and outsourced) and social impact. Following the LCA approach, there are three types of Life Cycle Costing (LCC: conventional or financial LCC, environmental LCC (E-LCC), and societal LCC (S-LCC) (Kianian *et al.*, 2019). While conventional LCC focuses on costs borne by two stakeholders: producers or users, E-LCC evaluates the costs associated with the life cycle of a product across multiple actors, using the same product system defined by LCA in the ISO 14040/44 standards (Swarr *et al.*, 2011). For example, Roffeis *et al.* (2018) analysed the life cycle cost of insect-based feed in West Africa, following the SETAC code of practice (Gluch and Baumann, 2004). Spykman *et al.* (2021) applied environmental and economic assessments (ecoefficiency) to insect production for food and feed, following Rödger *et al.* (2018) recommendations for E-LCC. Similarly to LCA, there is no specific standard LCC method which can be applied to the insect industry.

For those concerned with broader societal implications, S-LCC considers the costs borne by society as a whole (Hunkeler *et al.*, 2008). As sustainability becomes central to businesses and societies, social sustainability must integrate with environmental and economic pillars. However, a lack of consistency and methodology standardisation in Social Life Cycle Assessment (S-LCA) remains a challenge (De Menna *et al.*, 2018; Pollok *et al.*, 2021). Although protocols such as UNEP (2020) and the Product Social Impact Assessment Handbook (Benoît-Norris *et al.*, 2011) exist, further efforts are needed to streamline S-LCA methods. A recent advancement in the standardisation of S-LCA methods became the publication of ISO14075:2024 standard, aimed to set universal guidelines for social impact studies. So far in insect studies, application of S-LCA is very sporadic. Macombe *et al.* (2019) have analysed the social effects of industrial scale insect value chains in Europe, while there is still a need for more studies doing the social assessment related with insect production.

For LCA practitioners applying S-LCA, the most recent guidelines are available in the Product Social Impact Life Cycle Assessment (PSILCA) handbook and the Social Hotspot Database (SHDB). There are also tools like SOCA, an add-on for the ecoinvent database, which integrates environmental LCA, social LCA, and LCC into a comprehensive Life Cycle Sustainability Assessment. SOCA contains 70 social indicators spanning all processes within the ecoinvent database (Huertas-Valdivia *et al.*, 2020). These resources contain essential social indicators for different stakeholder groups. It is recommended that any S-LCA practitioner should rely on the newly established ISO standard and mentioned databases for the alignment of further studies and practices.

Despite quite a few active developments in insect LCA, LCC and S-LCA – there are no studies which would holistically consider all three aspects. It is expected and urgently needed for the viable fair comparisons with other product s on the market, that further studies rely more and more on PEFCR (Product Environmental Footprint Category Rules) and EF (Environmental Footprint) methods (Damiani *et al.*, 2022) as a first step for the standardisation and unification of the assessment approach.

8 Advanced data analytics

Classical data analysis methods rely on descriptive statistics, basic regression analysis, correlation analysis, basic hypothesis testing, etc. With advanced data analysis methods, we define the use of machine learning algorithms, deep learning techniques, natural language processing, advanced regression techniques (e.g. non-linear regression), methods, which are aimed to trace and define non-linear correlations between components. Compared to the other areas of insect research, there are only a few studies that relied on advanced data analysis methods for their research. It is necessary to differentiate the studies, which applied, adapted and developed tools for the improvement of insect production and processing (applied direction of research) and studies aimed for the improvement of research (more theoretical domain). However, in some cases, it is hard to draw a sharp line. Nevertheless, it is useful for the structure of the future studies to analyse the potential of advanced data analysis methods from this perspective.

A few application-oriented studies aimed for the development of Decision Support Systems (DSS). In modern days DSS are referred to as a set of related computer programs and the data required to assist with analysis and decision-making within a defined system boundary (e.g. organisation). While there are no established ways to design a DSS, following rules are rather

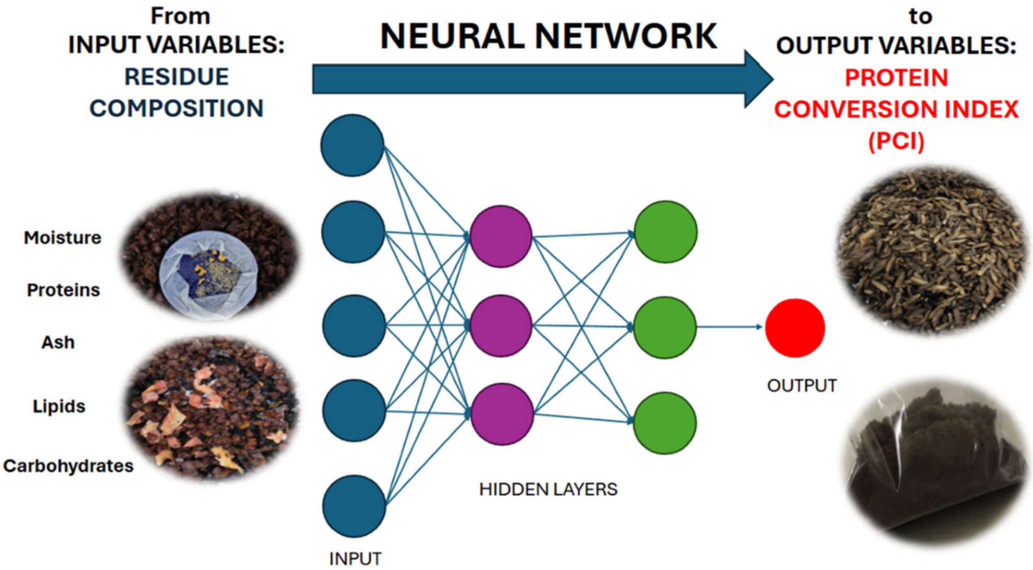

FIGURE 3 Example of general structure of the neural network construction for the production of protein yields based on the parameters of the input feeds (Vargas-Serna *et al.*, 2025).

classical: DSS should be utilised by the user following the requirements of a manual or instructions; DSS should include three main architectural components: the database (knowledge base), the model (algorithm of decision making) and the user interface; and DSS should be operated through inclusion of inputs and generation of outputs, followed by their verification through the decisions. Among the examples of DSS development could be DSS for sustainable insect production based on multi-objective optimisation of required parameters (Mouhrim *et al.*, 2022, 2023). Similar approach of creating DSS to improve the usability of ML algorithms targeting decoding and classification of cricket songs, along with their associated key weather variables (temperature and humidity) (Kyalo *et al.*, 2024). DSS often relies on the background data analysis of insect behaviours or their biometrical parameters with the application of relevant detectors (microphones, cameras, temperature and humidity meters, etc.).

The collection of experimental data through the application of some kind of sensors and digital recording of the data provides opportunities to operate continuous or discrete-continuous data throughout needed long periods of time without human supervision. Generation of big amounts of data provides opportunities not only for improved statistical analyses, but also for the application of machine learning algorithms (MLA). One of the examples (Vargas-Serna *et al.*, 2025) relies on artificial intelligence (AI) driven approach by using a structured two-layer neural network with four neurons in each hidden layer and one output neuron, employing logistic sigmoid functions in the hidden layers and a linear function in the output layer. Such a neural network allows the prediction of larval *H. illucens* protein yield based on the nutritional composition of the feed mixtures for larvae cultivation Fig. 3.

Further MLA as convolutional neural networks (CNN) can be applied for image recognition and segmentation for the identification of insects and their development stages (Bjerge *et al.*, 2019, 2022; Majewski *et al.*, 2022, 2024). Regression and Mask regression CNN require substantial sets of data (images in this case) for MLA training and verification. Recently there has been a lot of development in research aimed for the pollinating insect detection (Gharaee *et al.*, 2024; Sittinger *et al.*, 2023, 2024). Also, for some mass-produced insect species (e.g. mealworms, crickets, bees) this challenge can be solved by using already available databases (Papadopoulos *et al.*, 2024). Moreover, it is highly recommended to use the available databases for the testing of any AI tools before proceeding with the generation of own images to assure maximum complementarity and interoperability of data. It should be noted that use of unstructured and unlabelled images could lead to inconsistent results, therefore the of transfer learning models with softmax classifiers are recommended (Abeywardhana *et al.*, 2022).

Fast applications of advanced data analytical tools find their role in the automation of mass insect rearing (Kröncke *et al.*, 2020; Majewski *et al.*, 2022; Papadopoulos *et al.*, 2024), especially for the automatic control of environmental conditions and rearing substrate (Sabir *et al.*, 2020; Van *et al.*, 2022; Vo, 2020; Warta *et al.*, 2022). Most of the automation applications are associated with

T. molitor production, which might be associated with the relative disclosure of the insects to the tracing cameras compared to the other insects. However, there are also exception of application of Mask Scoring R-CNN for the image segmentation in cricket producing facility to identify and locate individual crickets in images automatically defining its features such as shape, colour enabling to derive the age, size, and health for automatic monitoring of insect growth ensuring stable production processes for delivering high quality products (Wenning *et al.*, 2022). Another exception is the development of bio-economic model of *H. illucens* production for the tracing of change of economic parameters associated with the changes of biological traits of species (Zaalberg *et al.*, 2024). Application of bioeconomic models, also considering the application of genetic improvement options, for the reflection in economic models is an approach to follow both in research and applied studies. Algorithms associated with automation or improvement of analysis are also applied in research activities for non-invasive determination of the size and growth dynamics of larvae (Majewski *et al.*, 2022, 2024), insects and their parasites detection (Bjerge *et al.*, 2019; Gupta and Homchan, 2021), insect counting and sex determination (Hansen *et al.*, 2022; Tao *et al.*, 2019).

Already classical in deep learning approaches and computer vision are application of CNN (convolutional neural networks – a type of deep learning network, based on self-learning via filter or kernel optimisation). Different types of CNN are a very useful tool when a pool of data (images, sound recordings, or other records) exist and can be used for algorithm training. CNN then can be used for object recognition, classification and segmentation in images and videos, language processing and recommendation developments, as demonstrated in insect studies as well (Bjerge *et al.*, 2019; Kyalo *et al.*, 2024; Majewski *et al.*, 2022, 2024; Wenning *et al.*, 2022).

Among the most applied algorithms we can highlight YOLO (You Only Look Once) of different versions (currently more than 10 versions), which are developed on the futures learned by a Deep Convolutional Neural Network (Darknet) and designed for a real-time object detection (Redmon, 2016). Despite the sequence of the versions, they differ in the application goals as they were often designed by different authors. YOLO is a single stage detector, able to generate the outcome with a single shot regression approach to identify objects bounding boxes and their classification in a single pass of a network. It is done in a short time and with a high level of accuracy. YOLO models are also very small and can operate on limited computing power. YOLO models can be applied mostly for object detection and determination in real time in set-ups with cameras, e.g. for detection of *T. molitor* (Papadopoulos *et al.*, 2024) or detection or tracking of pollinators (Bjerge *et al.*, 2022; Hansen *et al.*, 2022; Ratnayake *et al.*, 2021).

Other, less common in insect studies approaches include application of GMT (google teachable machine) for *Acheta domesticus* detection (Gupta and Homchan, 2021), watershed algorithms for insect image processing (Nawoya *et al.*, 2024), Support Vector Machine (SVM) for *A. domesticus* classification (Hansen *et al.*, 2022) and MobileNet Single Shot Detector (SSD-MobilenetV2) for object detection (Hansen *et al.*, 2022). These approaches have high potential for monitoring insect health and welfare in production systems, but such automated image analysis has merit also for improving high-throughput phenotyping systems (Laursen *et al.*, 2021) in the context of selective breeding of insects for food and feed (Hansen *et al.*, 2024).

Special attention for the application of advanced tracking mechanisms is given in the studies associated with bees and pollination. Thus, such algorithms as YOLOv4 and KNN in combination with linear regression analysis and background segmentation were applied for the analysis of honeybees such as in and out activities and pollination (Ratnayake *et al.*, 2021, 2023; Tu *et al.*, 2016).

9 Conclusions and outlook

In conclusion, designing reliable insect experiments requires careful consideration of several key principles: replication, randomisation, and local control, which ensure that results are statistically robust and representative. Standardising methods, reducing sources of variability, and ensuring precise characterisation of treatments are critical for producing comparable and reproducible results. Experimental designs, such as unchanging factorial designs, repeated measures, and change-over designs, each have specific advantages depending on the research objectives. Mixed-effects and generalised linear models joined with statistical expertise offer flexibility in handling complex data structures and predictive assurance. By adhering to these principles and methods, researchers can enhance the reliability and validity of their findings, contributing to the advancement of insect studies.

Microbiological and toxicological studies require comprehensive approaches involving microbial quantification, characterisation, and toxicological assessments address the challenges posed by environmental influences and rearing substrates. Microbial community analysis using sequencing technologies and advanced statistical tools offers insights into gut microbiota's role in supporting insect health and product safety. Similarly, toxicological evaluations using state-of-the-art chemical analyses mitigate risks from harmful substances like heavy metals, pesticides, and mycotoxins. Together, these methodologies enable informed risk management, fostering sustainable and safe insect production practices.

Behavioural modelling for mass produced insects requires often nuances statistical approaches due to the complexity of reproductive behaviours and zero-inflated count data. Hurdle models are particularly effective, separating zero and positive counts to better predict behaviours like mating frequency and oviposition. Nonparametric methods and advanced statistical tools, such as Kernel regression and generalised additive models, also address the challenges of non-normal, skewed, and heterogeneous data. These approaches are essential for capturing the variability influenced by ecological, demographic, and environmental factors, enabling more precise and biologically meaningful insights into the behaviours of insects produced for food and feed.

Future research in consumer studies should explore innovative data collection techniques, such as mobile surveys and online focus groups, to capture real-time consumer insights (Hackley, 2007). Additionally, the increasing importance of big data analytics presents opportunities for consumer researchers to leverage large datasets to uncover patterns and trends in consumer behaviour (Sitz, 2008). Furthermore, interdisciplinary approaches that incorporate insights from psychology, sociology, and behavioural economics can enhance the understanding of consumer decision-making processes (Miah *et al.*, 2022). By integrating diverse theoretical frameworks and methodologies, researchers can develop more comprehensive models of consumer behaviour that account for the complexities of the modern marketplace (Miah *et al.*, 2022). Future studies should also explore the role of consumer involvement in data collection and analysis, as this can significantly enhance the quality and applicability of research findings (Thach *et al.*, 2021). As the field continues to evolve, researchers must remain adaptable and innovative in their methodological approaches, ensuring that they effectively capture the nuances of consumer behaviour in an ever-changing landscape.

Sustainability studies, including LCA, LCC, and S-LCA, have evolved into critical tools for integrating environmental, economic, and social dimensions in insect production. Future developments require unified studies and guidelines. For practitioners, leveraging advanced tools of sustainability assessment and aligning with established standards and databases will enhance the rigor and scope of studies, enabling more comprehensive evaluations of insect-based systems.

The use of advanced data analysis methods in insect research, though still emerging, is critical for both theoretical advancements and practical applications. Decision Support Systems (DSS) and tools like CNNs and YOLO models demonstrate the transformative potential of automation and AI for optimising insect farming, behaviour analysis, production processes and selective breeding. Challenges such as data quality and consistency underscore the need for standardised approaches, including transfer learning and leveraging existing databases. Bioeconomic models further integrate biological and economic insights, enhancing decision-making frameworks. It is important to adopt advanced tools and maintaining methodological rigor to drive innovation in insect research, fostering sustainable solutions for food and feed systems. Quantum computing, though not yet widely applied in insect farming, holds great potential for optimising production and sustainability. Its ability to process vast genomic data and enhance environmental monitoring could streamline breeding programs and improve automated phenotyping efficiency. Additionally, regulatory challenges remain a barrier to the widespread adoption of insect farming in Europe. Technologies like blockchain and predictive analytics can support compliance by ensuring traceability, monitoring feeding substrates, and assessing potential contaminants, reinforcing food safety regulations.

The integration of both traditional and advanced analytical methods has proven essential in addressing the multifaceted challenges of insect production for food and feed. While traditional methods provide a robust foundation, the incorporation of machine learning, bioinformatics, and sustainability assessment tools marks a paradigm shift towards data-driven innovations.

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