Interreg North-West Europe

EUROPEAN UNION

ValuSect

European Regional Development Fund

Recommendations for *Guidelines on insect research*

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1. Introduction

This policy brief is written to **create awareness** on the need for guidelines on insect research. In times of a continuously rising world population and prosperity, the need for alternative and sustainable protein sources is high. Insects are considered one of the promising alternatives for, among others, food and feed applications; and therefore have gained growing interest from researchers worldwide. As this resulted in increased knowledge and many publications generated, it is now the time to implement **guidelines** and procedures on the practices of insect research. This is a very important step in order to be able to compare data of different experiments and publications and to join efforts for conducting research and, consequently, promoting the growth of this upcoming industry. In this policy brief, we suggest experimental and analytical methods that should be standardised; as well as information that should be always reported. Our suggestions are based on the authors' experiences and aim to serve as basis for guidelines on insect research.

2. Issue

As part of the Interreg NWE ValuSect project, it was aimed to define the knowledge gaps on insect production and processing, as well as to contribute to filling these gaps to provide insect industry stakeholders with relevant and practical information. In order to do so, the ValuSect consortium conducted several literature reviews, namely on substrates used for insect rearing, insect processing, nutritional value, functionality and potential hazards.
One of the challenges that came forward when conducting the abovementioned literature reviews, was the lack of standardisation on several insect research topics such as insect production and analysis. The differences in experimental designs, rearing techniques, lack of control diets, results reported and monitoring of parameters are limiting the comparison between the published studies and, consequently, how existing knowledge can be utilised in a more efficient way.

Insects are considered a promising and sustainable alternative protein source, and will most likely play a very relevant role in a future and more circular economy and sustainable society. However, the application of insects in food and feed is a relatively new concept in Western countries and, therefore, still in its infancy. Many research questions still need to be addressed in order to optimise the production and use of insects. Consequently, insect production and processing will become, most likely, an important field of study, in many different countries and institutions. It is clear that there is a great need of robust guidelines and protocols on insect experimental designs and analytical techniques.

2.1. Need for research protocols on insect production

As the insect industry is an upcoming and promising sector, it is key that guidelines on research practices are drawn up in order to compare data and harmonize research. This will enable to efficiently provide the sector with relevant data and promote its growth.

The growing interest on insect production practices has lead to an exponential increase of publications in recent years. However, the lack of information provided in these publications often hamper comparison among them. For example, it is known that the insect rearing conditions highly impact research results, but often these rearing conditions are not or only partly mentioned in conducted studies. Furthermore, for insect feed experiments, diverse methodologies have been applied to quantify the efficiency of conversion (e.g. feed conversion ratio, bioconversion efficiency, etc.). Since there are now numerous of methods described and consequently used among studies, comparison of data becomes quite challenging.

Also, often different studies implement different insect harvesting strategies and times (i.e. end of experiment). For example, some studies might use the 'appearance of first pupae/adult' as factor to terminate the experiment while others might use a fixed time (e.g. 20 days) or stagnation phase of the growth curve.

In addition, some studies exclude a control treatment, which not necessarily decreases the value of the study, however, it might limit the relevance to other studies (i.e. comparison).

2.2. Need for guidelines on insect analysis

Besides the need for standardization of insect rearing conditions, it is as important to harmonize appropriate analytical methods to determine the nutritional value of insects and their feed. Or at least, the used analytical techniques should be clearly mentioned with the necessary details to interpret the results. For example, crude protein content of insects and their feed can be determined by several analytical methods that have significant different results. Commonly used is Kjeldahl and Dumas technique, where the nitrogen content is determined and protein content is calculated from this with result using a Nitrogen to Protein factor. However, different factors are used (6.25 (standard), 4.76 (Janssen et al., 2017), 5.33 (Boulos et al., 2020). In case of their feed, it can be difficult to predict the right protein content as there are many other compounds present that contain nitrogen (e.g. chlorophyll in plants). Amino acid analysis can be a more precise technique, but the sample preparation method for this analytical technique has a great impact on the result. As amino acids need to be hydrolyzed in advance, some amino acids get destroyed depending on the used method. All these information about sample preparation and calculations need to be mentioned in studies to correctly interpret the data and compare data from different studies.

3. Progress

3.1. On standardization of insect production research

In 2020 Bosch et al. expressed the need for guidelines on the standardization of black soldier fly feed experiments (Bosch et al., 2020). With this publication, the authors created the first base of a protocol for black soldier fly research. In response, a work group bound to the EAAP congress consisting of partners active within the black soldier fly research and production sector has been established. Partners of this initiative drew up a protocol for black soldier fly feed experiments and performed a Ring test.

Meanwhile, as part of the ValuSect project, partners performed preliminary experiments to construct a standardised protocol for mealworm feed experiments. This protocol was drawn up internally for project purposes as different mealworm experiments had to be performed by different partners of the consortium. The protocol was tested, evaluated and adjusted by three ValuSect partners. Afterwards, the results were distributed to an initiative similar to the black soldier fly work group: a work group bound to the EAAP congress consisting of partners active within the mealworm research and production sector. This work group, in which several ValuSect partners take part, resulted in a more advanced protocol which is currently being revised through the performance of a Ring test (Berrens, 2021).

To compare data and harmonize research we suggest the more and detailed information should be reported when conducting insect research. This suggestion is general and should be further detailed for each insect species. Furthermore, as many parameters can be determined by different methodologies agreements should be made on the standardisation of the methods.

As part of the ValuSect project, a first step is made and we constructed a protocol for insect feed experiments including experiments with yellow mealworm, house crickets and migratory locusts. For yellow mealworms and house crickets this protocol was tested by 3 different partners, including a partner with minimal rearing experience. After interpretation of the results, the protocol was adjusted and is currently being formatted into a report that, as an outcome of the ValuSect project, will soon be published and serve as basis for guidelines on standardization of insect research. This protocol also includes guidelines on proper sampling and suggestions on the analysis of frass, insects and their substrate.

3.2. On standardisation of insect analysis research

To the best of our knowledge no standardization on insect analysis for research purposes has been performed yet. However, as outcome of the ValuSect project, a report will be published that can serve as basis for insect analysis standardization for sampling and proximate analysis.

4. Suggestions

The focus of these suggestions is on the inclusion of information and details rather than on rearing conditions, analysis methodologies, etc. The suggestion is sometimes clarified with practical examples, however, for the complete suggestions on conditions, techniques & methodologies (i.e. protocol on insects rearing and analysis for research purposes) the reader is referred to future ValuSect reports.

4.1. Insect production

Even for research purposes, we strongly suggest to standardise rearing methods and conditions as much as possible and modify those conditions only under study in the particular experimental design.

a) Before experiment

Guidelines should not only address the actual insect experiment, but also the preliminary phase. Often a parental colony is present from which the offspring will be used for experimental purposes. As this population is often domesticated for quite some time, information on 1) climate conditions (temperature, relative humidity), and 2) the basic rearing process (i.e. diet, feeding regime, day/night regime if relevant, oviposition duration, oviposition substrate, etc.) should be reported. Populations domesticated for some time often contain genetic selected and isolated insects. This often results in adaptations of the population to their environment (e.g. to a certain control diet). Even though published data on genetic differences between insect strains are limited at this point, there are some indications (including our own experience) that the origin of insects can affect experimental results (Zhou et al., 2013).

Exception to previous suggestion is the use of wild populations. In this case, data on location of collection should at least be mentioned. When working with offspring of wild populations, incubating conditions should also be reported. When using insects of breeders, the company should be mentioned and if possible, their basic rearing process.

After harvesting insect eggs, we strongly suggest to incubate the eggs until hatching and, if relevant to the research scope, to include a nursing phase. The duration of the nursing phase will depend on the insect species (development time), however, should cover the critical stage of the insects. Meant by this is the fragile phase of insects, often referred to as neonatal stage, where insects are vulnerable for manual handling and there is a high percentage of natural insect loss. A nursing stage should especially be included when performing feed experiments. At this stage the insects need an optimal diet and are not yet able to thrive on low-value substrates. Furthermore, as the insects are not yet able to process large amounts of feed, the substrate might get mouldy, affecting the results of the experiment. We suggest the following:

- the nursing phase of black soldier fly larvae should last 5 days
- the nursing phase of yellow mealworms should last 4 weeks
- the nursing phase of house crickets should last 2 weeks

- the nursing phase of migratory locust should last 1 week

These suggestions are based on the vulnerability of the young insects and the possibility to perform subsampling during the experiment (manual handling and weighing). After nursing the experimental population, the experiment can be set up.

Egg incubation conditions should be reported, as well as the nursing conditions such as: climate conditions, diet (including amount of water if relevant) and feeding regime.

In addition, it is important that the origin of the diet(s) used is (are) mentioned in the study, as well as the ingredients and proximate analysis (refer to AOAC methods for its determination, it should include at least: moisture, crude protein, crude fat, crude fibre, ash, and pH), pre-treatment specifications and the storage method, especially when performing feed experiments with insects.

The table below summarizes the minimal information on 'before start of the experiment' we suggest should be mentioned in insect research.

Before experiment		
Parental population	**Egg harvest**	**Nursing phase**
Origin: Company or location	Harvesting method used	Rearing conditions: • Temperature • Relative humidity • Day/night regime •
Rearing conditions: • Temperature • Relative humidity • Day/night regime • Light source (if relevant)	Incubation • Temperature • Relative humidity • Day/night regime	Diet: • Origin • Ingredients • Pre-treatment • Storage conditions and duration • Proximate analysis with specification of techniques
Diet: • Origin • Ingredients • Pre-treatment • Storage conditions and duration		Rearing process: • Duration of nursing phase • Feeding regime • Population density

• Proximate analysis with specification of the techniques		
Rearing process: • Oviposition substrate • Density • Oviposition duration (spread on age eggs)		

b) During experiment

Especially during the experiment, the rearing conditions should be standardised per insect species to be able to compare among studies, if this does not affect the scope of the research. For example, applying a temperature of 30 °C and 60% relative humidity for the rearing of yellow mealworms.

Rearing conditions such as **temperature, relative humidity, day/night cycle and source of lighting (**if relevant) should be reported.

As density often impacts the insects performance, we strongly suggest that this is standardised per species based on the optimal conditions. Applying unsuitable densities during the experiment might affect results. For example, biocontrol due to overcrowding and competing for space, especially in cannibalistic species such as house crickets and locusts increases mortality rates (unrelated to the studies scope). Furthermore, the metabolic heat produced by the insects, especially the case with species that thrive in their substrates (e.g. mealworms, black soldier fly larvae), might impact the research data.

In addition, the amount of feed given should be adapted by the applied density. For optimal application, the ideal feeding regime (amount of feed/insect/day) of the insect species should be known. As for some species additional research on this topic is still needed, researchers often apply an *ad libitum* feeding strategy. However, it should be noted that *ad libitum* feeding affects conversion rates and efficiencies. Furthermore, when applying an *ad libitum* feeding strategy, we strongly suggest to monitor and report the exact amount of feed given during the experiment. This also includes the supplementation of moisture sources such as water as also this impacts the results.

Different parameters are monitored during insect research experiments and are important for the good interpretation of the results. Therefore they should be as complete as possible, depending on the research question. We strongly suggest to construct a larval growth curve and for this perform subsampling at least once a week. Subsampling should be done with several individuals, however, this depends on the scale of the experiment. For example, when performing mealworm experiments on pilot scale (i.e. 60 x 40 cm rearing crates), we strongly suggest to include at least 100 larvae in the subsample. This subsample should be taken randomly after mixing insects and substrate. Furthermore, it is very important that every individual insect present in the subsample is counted.

During the ValuSect experiments, we experienced issues with the reliability of subsampling and therefore the method should be standardised. The method suggested by the ValuSect consortium will be published later more in detail in an optimised standard insect rearing protocol for feed experiments per insect species.

For insect feed experiments we suggest monitoring the following parameters:

- insect growth and development by larval growth curve
- feed conversion ratio (dry/wet), (additionally wet and dry)
- and/or bioconversion efficiency
- mean individual end weight
- insect yield (total end weight of insects per crate)
- survival rate (%)
- insect proximate analysis

As previously mentioned, diverse methodologies have been applied to quantify parameters such as insect conversion. To our opinion, the most suitable method should be standardised in insect feed experiments to be able to compare data among studies. To provide an example, it should be standardised whether the larval final weight will be determined by 'mean maximal weight' or 'mean final weight', and, conversion of insects will be determined by 'FCR', 'bioconversion efficiency', 'efficiency of conversion of ingested food (ECI)', 'Waste Reduction Index', several or all. Formulas for calculating such parameters should be reported.

It generally is advised to perform at least four replicates during the insect experiment to reduce standard deviations and increase the value of the research (Bosch et al., 2020). However, more replicates (e.g. 6) might be suitable depending on the scope of the study and the scale. Especially when the rearing experience is limited, we suggest performing 6 replicates.

Including independent replicas might be relevant. With this is meant time independent replicas (repeating the experiment at another period), as well as population independent replicas (e.g. other research group). When performing the independent replicas, a control must be included as an internal standard to avail for potential variations due to starting population, climate conditions or other factors out of the control of the researcher. For the latter, comparison must be possible and the entire experimental design and rearing process must be reported. Note that data from experiments performed with a single insect strain/population might not be applicable globally.

The table below summarizes the minimal information on 'during experiment' we suggest should be mentioned in insect research.

During experiment		
Diets used	**Rearing conditions & process**	**Parameters**
Origin	Temperature	Conversion • Method • Calculation
Ingredients	Relative humidity	Growth • Method of subsampling • Growth curve
Pre-treatment • Method • Equipment	Day/night regime Light source (if relevant)	Mean individual end weight
Storage • Conditions • Duration	Amount of replicates	Total yield
	Feeding regime	
	Density	Insect survival rate

c) End of experiment

Before the insects are harvested, a starvation period should be included for analysis purposes as it is important to interpret the analytical results when determining the impact of the feed on the insect composition. For example, minerals accumulation is limited in insects, but higher mineral content can be measured when insects are not starved. When guts are still full, this content is analysed as well (gut loading).

The end of the experiment, i.e. harvesting time, often differs among studies. This makes it tough to compare parameters such as insect end weights and growth curves. Defining the end of the experiment is challenging, as we experienced ourselves during the ValuSect project, and it depends on the insect species. Different methods used vary among studies, for example:

- a fixed time
- time insects run out of feed
- certain percentage of pupae/adults present (e.g. 50%)

- time first pupae/adult appears

All methods have pros and cons, but as the percentage of pupae/adults is very difficult to determine objectively, we would not recommend this as standardised harvesting time.

The time insects run out of feed can only be used as harvesting point when the FCR and feed requirement is optimised and proved with a various amount of studies, as for example with black soldier fly larvae.

Using a fixed time to end the experiment is the easiest method, however, this risks of ending the experiment too soon when insects are still in their exponential growth. For instance, one population might have lower development time than another, making comparison once again difficult. Furthermore, the slightest changes in environmental conditions might impact on the insect development time.

The same applies to the 'time first pupae/adult appears' as often there is some spread on the age of the insects, affecting the insect growth curve.

Taken all this into account, it seems choices have to be made on the standardisation of the harvesting time. We suggest the following:

Hemi metabolic insects (e.g. crickets, locusts): we would like to suggest to end the experiment on a fixed time, provided that the rearing cycle of the insect is well known and stable for that population (e.g. harvest 2 days before mean transformation to adults). Holometabolic insects (e.g. mealworms): we suggest to end the experiment when the first pupae appear in the control treatments. The control treatments, as during feed experiment with low-value streams insects, might not pupate due to (for example) insufficient nutrient availability.

During the harvest of the insects, samples for further analysis must be taken. For this, the reader is referred to 'sampling methodologies' later in this document.

The table below summarizes the minimal information on 'end of experiment' we suggest should be mentioned in insect research.

End of experiment
Harvesting time: • Duration of experiment • Method (i.e. fixed time, first pupae present, etc.) • Starving period (if applicable)
Harvesting • Method • Materials used (if relevant)
Insect sampling low insect samples are processed and stabilised for storage (freezing, grinding, vacuum pack, tc)

4.2. Insect analysis

Insect proximate analysis should include at least:

- crude protein content,
- crude fat content (or ether extract),
- dry matter content,
- crude ash content,
- chitin or crude fiber content.

For each of these parameters, the used analytical technique and sample preparation must be mentioned in order to correctly interpret the results. As there are different techniques to determine each of these parameters and techniques have big influence on the results.

a) Sampling methodologies

When sampling insects for analysis, the amount of sample should be determined. For this it should be known whether the analysis will be performed in duplicate, or ideally, in triplicate. We suggest an amount of at least 300 g fresh sample for proximate analysis in triplicate. Samples must be dried and homogenized prior to analysis. The required pretreatment is related to the component that needs to be determined and the analytical technique. For proximate analysis, insects can be air-dried and grinded (to pass a 1mm screen). The drying technique or temperature is dependent on the component to be analyzed. For example, drying temperature for determination of fibers (or chitin) should not exceed 60°C to preserve fibers when using extraction bags that must retain the fibers during analysis. When analyzing more fragile components like amino-acids or vitamins, other drying techniques than hot air drying must be applied (e.g.freeze-drying) in order not to destroy these components. Dried samples should be stored in air-tight containers in the dark at room temperature or freezer prior to analysis. Again, the storage conditions are related to the component that needs to be determined and the analytical technique that is used. Information about the sample preparation and related analytical protocols that were used are important information to interpretate results and must therefore be mentioned.

Since some insects such as mealworms are cultivated in their substrate, it is important they are washed to remove contaminations such as frass and feed left-overs. The protocol applied to remove non-desired components needs to be harmonized (sieving, washing out, etc.). For example, washing in lukewarm water, next rinsing with demineralized water and drying with a paper towel.

b) Pretreatment for analysis

Pretreatment can include one or several steps until the sample is ready to be analyzed.

1) First the insects need to be killed before analysis can be performed. Killing techniques as blanching or freezing can be used. The temperature and duration of the process must be mentioned.

2) The insects then need to be dried. Different techniques are available for drying such as oven drying, microwave drying, freeze drying etc. Temperature as time are here also important parameters which need to be clarified (specifically for microwave drying, the wattage must be mentioned). For freeze drying the vacuum pressure for the process is an important parameter.

3) When the samples are dry, homogenization of the sample is necessary to perform correct analysis. It is recommended that the samples are milled to obtain a powder with a regular particle size.

4) Finally a separation step can be include depending on the further analysis of the insects. This can include filtering, pressing, enzymatic as isoelectric precipitation etc.

Since all these steps can be performed by many diverse processes, it is recommended to mention in detail how the starting material, before analysis, was processed and stabilized for storage (e.g. boiling, freezing, grinding, vacuum pack, etc). Processing parameters need to be reported in detail, to ensure that the same procedure can be replicated by any other researcher.

Unless the scope of the research is to determine the impact of any step in the pretreatment on the final product, it is recommended to always use the same protocol to minimize the variation on the results. This will apply to independent trials, in order to avail for better comparison.

c) Analysis practices

On our view, it is suggested to use AOAC methods since these are followed consistently for proximate analysis, fatty acid profile and amino acid profile. However, if this method does not allow to provide the required data, a different approach can be used. It must be noted that the methodology should be described in detail in order to correctly interpret and compare data among studies.

In case the AOAC methods are not followed, we recommend mentioning the following parameters for each specific method

1) When determining the dry matter and ash content, it is important to mention the temperatures at which the analysis are carried out as well the period for which the samples are kept at these temperatures.

2) For the determination of the crude protein content there are different techniques which could be used. It is recommended to always mention the method used along with the N to P factor used to calculate the results. If any specific preparation of the samples is required, this should also be clearly indicated.

3) The same applies to the determination of the crude fat content with the exception of the N to P factor.

4) When determining the chitin content of the insects, the technique used must be mentioned as well as the pretreatment of the sample. Specific parameters such as drying temperature and time should be mentioned as high temperatures can affect the fibers.

The table below summarizes the parameters on proximate analysis we suggest should be mentioned in insect research.

Parameter
Dry matter and ash • Time and temperatures used
Crude protein content • Technique that was used (Kjeldahl, Dumas, Amino Acid Analysis, …) • Related factors to calculate results (e.g. N to P factor) • Sample preparation (sample volume for Kjeldahl/Dumas, hydrolysis protocol for Amino acids)
Crude fat content • Technique that was used (extraction, infrared, …) • Sample volume • Sample preparation (grinding, hydrolysis, …)
Chitin content • Technique that was used (manual extraction, fibre system, …) • Sample preparation (drying temperature, defatting, …)

d) N to P ratio

It needs to be discussed and agreed what conversion ratio is the right one to be employed when converting nitrogen content into protein content.

Several published papers made reference to a 6.25 ratio, which is usually employed for mammal origin meat. Recent investigation, by analysing carefully the amino acid profile of several insect species, have highlighted that this ratio is an overestimation of the actual value, and other ratios are suggested (Boulos, Tännler et al. (2020)). After evaluating the amino acid profile of *T. molitor*, *A. domesticus* and *L. migratoria*, it was observed that the right pure protein conversion factor (K_A) was of 5.75, 5.51 and 5.49 respectively; meanwhile the nitrogen-to-protein conversion factor (K_p) was of 5.41, 5.25 and 5.33. These authors recommend using an average value of 5.33 and 5.60 for Kp and K_A respectively.

Similar approach needs to be taken when analyzing the protein content in the substrate. Depending on the substrate compositions, N to P ratio may change depending on where the proteins are coming from animal origin, plant, fungi, etc.

e) Digestibility

Since digestibility is becoming a very important factor for insect-based products, it might have to be considered to include this in the standard analysis. It is suggested to follow the methodology described by Brodkorb et al. (2019). This *in vitro* protocol was revised and updated by a multidisciplinary team, aiming to provide a standard protocol to be used in any research center or laboratory.

5. Conclusions

In conclusion, it is clear that there is a need on standardisation of insect research in order to compare data and harmonise research, which can be further transferred to industries and stakeholders.

This is an important step for supporting the growth of the upcoming insect industry. Standardising research to some extent will not be an easy task and will need to consider inputs from many parties active within the entire sector, from research to production and processing. The challenge lays on the lack of data, and that emerging research topics still need to be explored. After all, the insect industry is still in its early stages.

However, as insect research gained a lot of interest lately, and is expected to increase, the time to construct guidelines is now. We would like to ask to consider the information in this policy brief and include this in insect research. We aim for our suggestions to serve as a base for constructing procedures in initiatives where agreements can be made.

6. References

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What is ValuSect?

ValuSect is a project funded by Interreg North-West Europe. The ValuSect consortium will improve the sustainable production and processing techniques of insect-based products and transfer developed knowledge to agri-food businesses in North-West Europe.

Since March 2021, the project extended its focus to the insect feed sector.

Associated partners

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