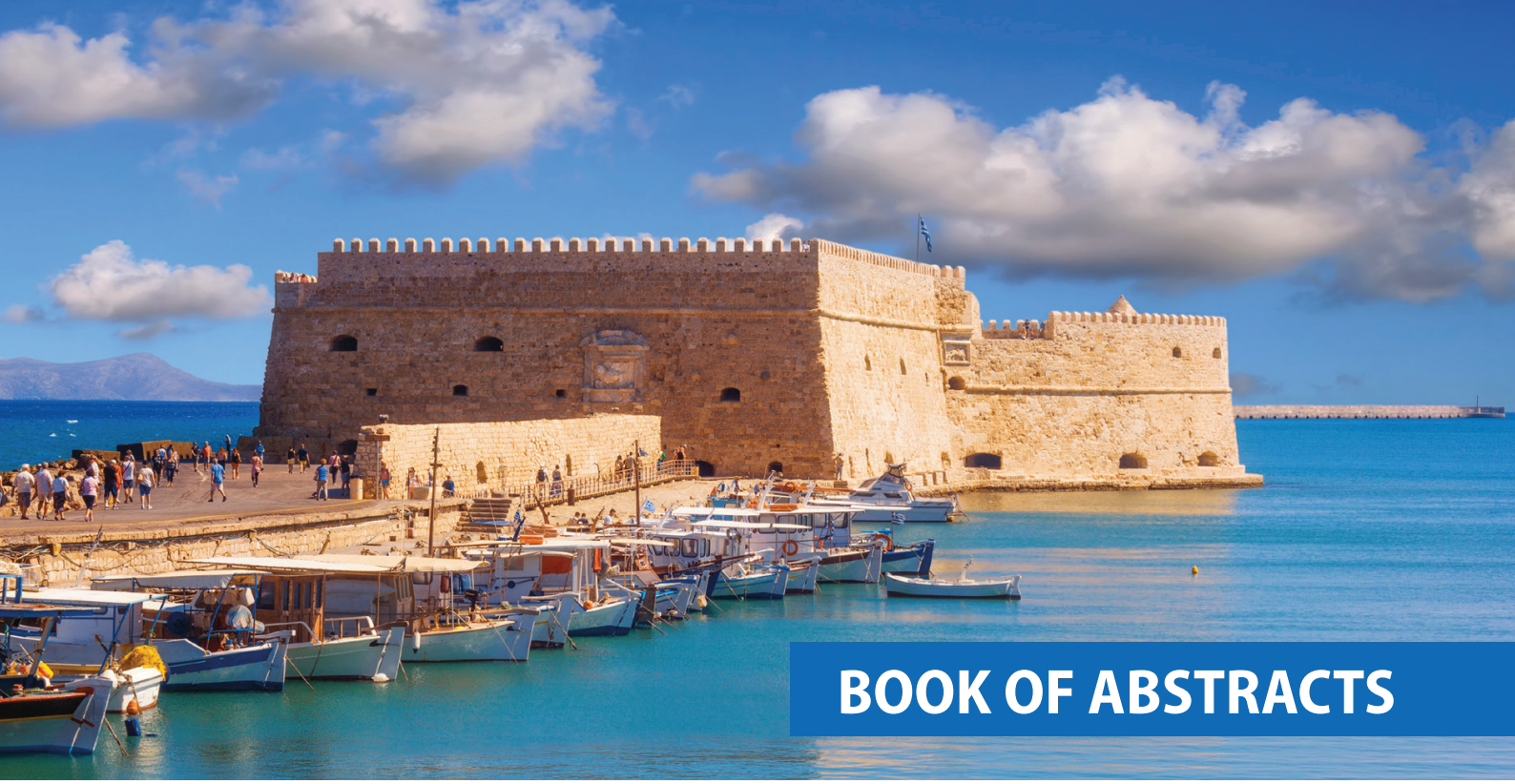
Asymptotic Estimated Digestibility, a new indicator to overcome challenges related to faeces collection and ingesta quantification in Hermetia illucens larvae

J B Guillaume, S. Mezdour, Frédéric Marion-Poll, Cécile Terrol, Philippe Schmidely



BOOK OF ABSTRACTS

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P374. Larval development of the superworm *Zophobas morio* (F.) (Coleoptera: Tenebrionidae) on substrates enriched with functional ingredients of aromatic and pharmaceutical plants of the Greek flora

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The interest in utilizing insects as a source of food and feed has been rapidly increasing. The inclusion of bioactive compounds and active ingredients in insect diets has been identified as a potential key factor for enhancing their growth and performance. The current study aims to investigate the growth and development of *Zophobas morio* (F.) (Coleoptera: Tenebrionidae) larvae, when fed on substrates enriched with functional ingredients derived from aromatic and medicinal plants of the Greek flora. The study evaluated the potential of wheat bran supplemented with two mixtures (A and B) at varying concentrations (10%, 20%, and 30%) as a substrate for insect feeding. The control group was provided with unenriched wheat bran. Mixture A comprised post-distillation residues obtained from various aromatic plants such as oregano, thyme, sage, and rosemary, as well as industrial cannabis, linseed oil, rock samphire, and olive paste by-product. Mixture B consisted of Mixture A augmented with essential oils derived from the aforementioned aromatic plants. Enrichment of the substrate with 20% of mixture A positively affected the development of *Z. morio* larvae and led to higher final larval weight compare to the control. In the case of mixture B, all enrichment rates resulted in higher final weight. The objective of our findings is to facilitate the utilization of insects as a source of food and feed by augmenting their feed with functional ingredients possessing favorable characteristics.
This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (Project Code: T2ΕΔΚ-02356, Acronym: InsectFeedAroma).

Keywords: circular economy, edible insects, insects as food and feed, insect protein

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P375. Asymptotic Estimated Digestibility, a new indicator to overcome challenges related to faeces collection and ingesta quantification in *Hermetia illucens* larvae

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Black soldier fly larvae (BSFL; *Hermetia illucens*) can quickly convert organic substrates into body proteins and lipids. Due to its high conversion efficiency, this specie has gained significant attention in the field of insects as feed and food. However, there is a lack of understanding of the digestive processes underlying this potential. The main challenges in assessing digestibility in BSFL are accurate feed intake measurement and proper faeces collection, as larvae feed and excrete in the same moist substrate. In this study, a new indicator called Estimated Digestibility (ED) was introduced, which measures the difference between distributed feed and frass macronutrient weight, divided by macronutrient weight in distributed feed. ED was evaluated at increasing larval densities to ensure complete feed ingestion and frass free from refused feed. Initial diet and frass samples were analyzed for dry matter (DM), starch, nitrogen, ether extract (EE), neutral detergent fibre, acid detergent fibre, acid detergent lignin, ash and energy. The results showed a sigmoidal pattern for ED of all fractions except fibres, with asymptotic ED of 80.3±1.3% for DM, 99.0±2.3% for starch, 78.6±1.1% for nitrogen, 95.3±1.5% for EE, 58.4±1.0% for ash and 80.6±1.2% for energy. This new indicator is a way of measuring digestibility in insects living in their feeding substrate. It offers insight into the digestive efficiency of BSFL.

Keywords: insect nutrition, alternative protein, insects as feed, macronutrient, density, Diptera

P376. Black soldier fly as a tool for the valorization of tomato waste

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The tomato is one of the most developed horticultural products in Italy and the tomato industry, particularly cannery industry, with 2.5 M tons/y produces enormous quantities of waste for which safe and cost-effective disposal must be developed. In particular, the re-use of washing and sorting residues, consisting of green leaves and stems and defective raw tomatoes (unripe, green or damaged), which represent 2-3% of the weight of the starting material, has been little studied.
In this work, tomato production residues were used as a substrate for the growth of larvae of *Hermetia illucens* (Diptera: Stratiomyidae), also known as the black soldier fly (BSF), that can grow on numerous organic substrates, converting them into valuable larval biomass rich in proteins and lipids. For this aim, different types of tomato wastes, such as stem, red or green tomatoes, leaves, seeds and peeling waste, were fed to groups of 500 BSF neonate larvae, with three replicates per each experimental trial. Growth parameters, bioconversion and substrate reduction indexes were then evaluated, and the larvae were analyzed to assess their final chemical composition. Results highlighted the ability of BSF to grow on the tomato waste, particularly those consisting of peeling waste (peel and seeds), or where tomato fruits were present in a higher percentage. In order to re-use waste biomass to obtain high added-value products, also in line with the EU's plan on the circular economy, tomato waste deserves further investigation for its exploitation for the production of BSF larvae.

Keywords: edible insects, *Hermetia illucens*, circular economy, vegetable waste

Digestibility in *Hermetia illucens* larvae: getting over faeces collection and ingesta quantification issues

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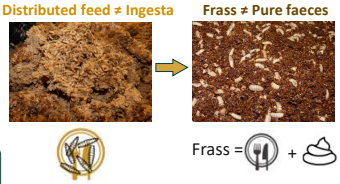
Background

- **Black soldier fly larvae** (BSFL; *Hermetia illucens*) can quickly convert various **organic substrates** into body **proteins** and lipids. Due to its **high conversion efficiency**, this species has gained significant attention in the field of **insects as feed and food** [1].
- BSFL fed diets with the same crude protein and carbohydrate contents but formulated with different ingredients show various performances [2], possibly due to different **digestive efficiencies**. This highlights the need to obtain digestibility values for **accurate diet formulation**.
- Digestibility calculation involves a **mass balance** approach based on **ingested feed (ingesta) and associated faeces** (Eq. 1). Accurate ingesta measurement and proper faeces collection in BSFL are challenging because **larvae feed and excrete in the same moist substrate**.

Equation 1

$$Digestibility = \frac{Mass\ of\ ingesta - Mass\ of\ faeces}{Mass\ of\ ingesta}$$



How can we measure digestibility in BSFL conversion systems?
Two methods (A and B) will be presented

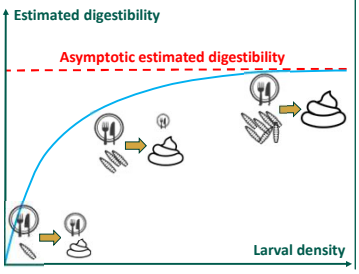
Materials and methods

A

- **Method A**: measuring **Estimated Digestibility** (ED; Eq. 2), calculated through a **mass balance between distributed feed and residual substrate** (frass).
- ED of dry mass (DM) was measured at increasing larval densities (0 to 29 larvae/cm²), after a **fixed feeding time**. We hypothesized that **high larval density** would result in **complete ingestion** of distributed feed and that **asymptotic ED** would reflect the total digestion potential of BSFL and their microbiota.
- 7-day old BSFL from Agronutris were fed 420g of fresh substrate. Trials were performed in 17x11x7cm containers in climate-controlled conditions (28°C, 75% RH, L12:D12).
- This approach was performed on chicken feed, discarded potatoes and corn gluten feed.

Equation 2

$$Estimated\ Digestibility = \frac{Mass\ of\ distributed\ feed - Mass\ of\ frass}{Mass\ of\ distributed\ feed}$$

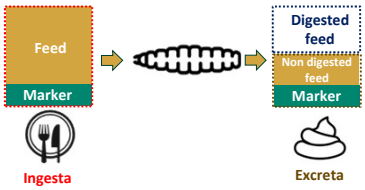


B

- **Method B**: addition of an **indigestible marker** (chromic oxide, Cr_2O_3) in the feed in order to calculate **Approximate Digestibility** (AD; Eq. 3). This method has been extensively used in livestock and other insect species [3].
- 200 eleven-day old BSFL were fed 400g of fresh substrate with **1% Cr_2O_3** (%DM). After 3 days, larvae were removed from the substrate, rinsed and put **in an empty container to let them defecate** for 24h. Excreta was collected by dilution with distilled water and a pipet, followed by water evaporation. Marker concentration in excreta was measured by **colorimetry** (540nm) after complete oxydation.
- This approach was performed on chicken feed, discarded potatoes, corn gluten feed, wheat bran and wheat distillers grain.

Equation 3

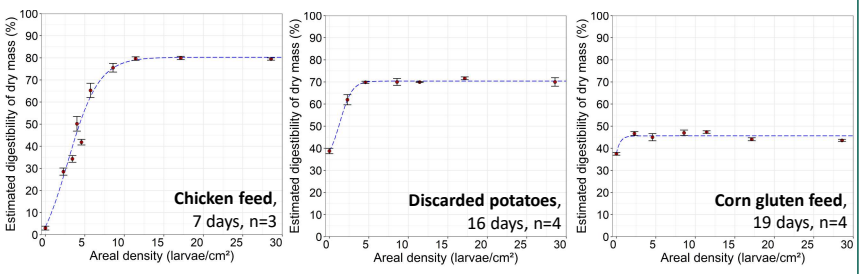
$$Approximate\ Digestibility = \frac{Marker\ conc^{o}\ in\ excreta - Marker\ conc^{o}\ in\ ingesta}{Marker\ conc^{o}\ in\ ingesta}$$



Results

A

- In **chicken feed**, all containers were sieved after 7 days of feeding. ED of DM increased with larval density following an **asymptotic trend**, up to a maximal value of **80.3±1.3%** (mean ± standard error).
- Asymptotic ED of **starch** (99.0±2.3%), **nitrogen** (78.6±1.1%), **ether extract** (95.3±1.5%), **ash** (58.4±1.0%) and **energy** (80.6±1.2%) were also assessed. Further details on chicken feed results have been published [4].

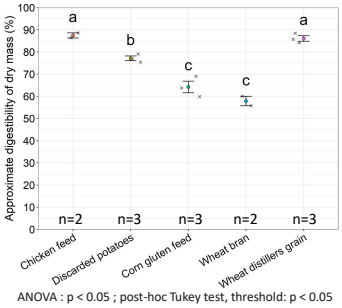


- In **discarded potatoes** after 7 days of feeding, **the frass was too moist and sticky** to properly separate it from the larvae. Feeding time was extended to **16 days** to allow proper evolution of frass texture. ED of DM in discarded potatoes increased with density but the asymptote was reached at lower densities than in chicken feed, presumably because longer feeding time allowed low-density containers to achieve similar digestion level as those with more larvae. **Asymptotic ED of DM was 70.4±0.7%.**
- The same issue with frass texture was observed in **corn gluten feed** and feeding time was extended to **19 days**, leading to low ED differences between densities. Slight decrease in ED of DM at high density is probably an artefact resulting from the initial inclusion of more residual frass with starter larvae at the start of the experiment. **Asymptotic ED of DM was 45.6±0.5%.**
- Asymptotic ED of **wheat distillers grain** was also explored, but could not be determined due to **low survival and start of pupation** (*i.e.* end of feeding) before frass texture allowed proper separation of the larvae.

B

- AD of DM determined with 1% Cr_2O_3 was 87.5±1.2% in chicken feed, 77.1±1.1% in discarded potatoes, 64.2±2.6% in corn gluten feed, 57.9±2.1% in wheat bran and 86.1±1.2% in wheat distillers grain.
- AD of nutrients such as starch or proteins could not be determined because **too little excreta was collected**.

Excreta left by 200 larvae after 24h (the green color is due to Cr_2O_3)



ANOVA : $p < 0.05$; post-hoc Tukey test, threshold: $p < 0.05$

- **AD of DM (Method B) was higher than asymptotic ED of DM (Method A) in all diets investigated.** A possible explanation is that, given their inability to ingest too large particles, BSFL might **exclusively consume the semi-liquid phase** of the diet containing all the marker, leading to an overestimation of AD. This is particularly likely in corn gluten feed which contained large maize pericarp particles.
- **The digestibility order remained the same in both methods**: chicken feed > discarded potatoes > corn gluten feed. These findings are consistent with the notion that chicken feed represents a highly effective formulated diet, discarded potatoes and wheat distillers grain are rich in digestible carbohydrates, while corn gluten feed and wheat bran have higher fiber content.

Conclusion

	A	B
Strengths	• High quantity of frass is collected, allowing for measurement of **ED of various nutrients** (DM, starch, nitrogen, specific amino acids or minerals, etc).	• Only considers digestion occuring **in larval gut**. • Easily **repeatable** on different substrates.
Weaknesses	• Asymptotic ED measurement requires total ingestion of substrate: **impossible in low-performing diets** (mortality, sticky frass, etc)→ Working on composed diets could allow for total ingestion. This approach would require to check additivity of ED. • Separation of frass and larvae is **time-consuming** at low-density or in low-performing diets. • Considers overall digestion by **both larvae and microbes** in the substrate. • In diets requiring extended feeding time to achieve non-sticky frass, **microbial digestion might continue** even after complete ingestion by BSFL, potentially resulting in an overestimation of asymptotic ED. The reliability of comparing asymptotic ED of diets with different feeding times could be questioned.	• Cr_2O_3 quantification requires **toxic chemicals** → Less toxic indigestible markers could be used. Titanium dioxide has recently been successfully used for AD determination in BSFL [5]. • **Inadapted for heterogenous substrates** or with large particles: risk of feed selection by larvae (marker concentration in ingesta ≠ marker concentration in substrate). • Excreta collection is **time-consuming**. • **Small quantity of excreta collected**: difficult to assess AD of various nutrients → The excreta collection procedure could be refined (*e.g.* using more larvae or longer gut-emptying period). However, these changes may come with new biases such as increased risk of coprophagy or microbial degradation of samples, leading to overestimation of AD.

Two methods have been proposed to assess digestibility in BSFL. These results provide insight into the digestive efficiency of BSFL and lay the ground for diet formulation based on digestible instead of crude nutrient contents.

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