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Feed, faeces and frass: getting over issues related to digestibility assessment in black soldier fly larvae

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Food and Feed for the Future

ISARA - Lyon, 1 September 2023

WORKSHOP PROCEEDINGS

A workshop sponsored by the OECD Co-operative Research Programme: Sustainable Agricultural and Food Systems



Food and Feed for the Future

Lyon, 1 September 2023

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and biogas from dairy cow faeces. It can be concluded that in the experimental groups, approximately 15.2% and 14.4% on a fresh weight basis and 11.7% and 10.9% on a dry weight basis more methane and biogas were generated compared to the control group. Similarly, high yields of methane and biogas were recorded when taking into account the content of dry organic matter in the faeces - on average about 13.1% and 12.3%, respectively. These are significant values also because the dry organic matter content in the experimental samples was more than 1.5% lower than in the control. Therefore, it can be assumed that the use of Rumitech in cow nutrition improved dietary digestibility which increased the efficiency of the use of faeces organic matter for biogas production. Project CCCfarming National Centre for Research and Development (SUSAN/II/CCCfarming/03/2021).

Feed, faeces and frass: getting over issues related to digestibility assessment in black soldier fly larvae

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Black soldier fly larvae (BSFL; *Hermetia illucens*) are one of the most promising species in the field of insects as food and feed. They are rich in proteins and lipids, can feed on a wide range of organic substrates, and have a high feed conversion efficiency. However, the mechanisms underlying feed conversion in this species remain poorly understood. BSFL live in their feeding substrate where non-ingested feed and faeces mix and form a material called 'frass.' Thus, digestibility studies are particularly hindered as ingesta can not be quantified, and it is impossible to collect pure faeces. The present study

presents and evaluates two approaches to tackle these issues. Our first approach relies on estimated digestibility (ED), calculated on distributed feed and frass weight. This assumes that, for the same feeding time and amount of feed, working with increasing larval densities would ultimately result in an asymptote of ED, indicating the maximal digestible fraction of the tested feeding substrate. The second approach, approximate digestibility (AD), is based on a usual method to study digestibility in insects or in conventional livestock species and requires the use of an indigestible marker (chromic oxide) incorporated into the tested feeding substrate. Results of these two approaches are compared for three feeding substrates (chicken feed, discarded potatoes, and corn gluten feed). Strengths and weaknesses of both methods are also discussed. This work provides valuable insight into the feed efficiency of BSFL and lays the groundwork for diet formulation based on digestible instead of crude nutrient contents for this species.

Greenhouse gas emission from livestock production in Poland and its impact on climate change – discussion of the farmers awareness survey

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The aim of the survey was to present information on the awareness of Polish farmers in terms of using available technologies to limit the negative impact of animal production on the environment. The survey was carried out on 44 farms in Greater Poland. Each one consisted of 87 open and closed questions which were divided into the following groups: farm characteristics, farmers experience, knowledge of measures to reduce greenhouse gas emissions, agricultural practices, knowledge of techniques to reduce ammonia emissions, support for environmental protection, general knowledge about greenhouse gas and ammonia. Based on the conducted surveys and interviews, it can be concluded that the awareness of Polish farmers about the risks arising from the above-mentioned phenomena is common but still insufficient. Most of the respondents knew about the existence of the phenomena



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

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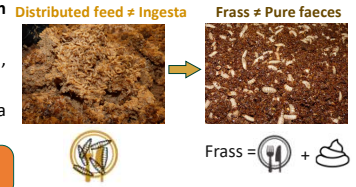


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Background

- **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**.

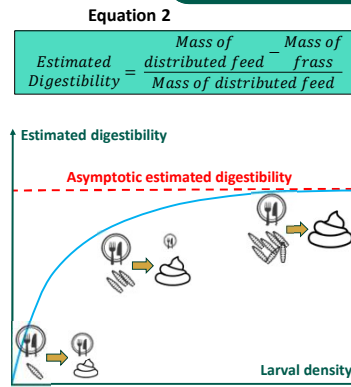


Equation 1
$$\text{Digestibility} = \frac{\text{Mass of ingesta} - \text{Mass of faeces}}{\text{Mass of ingesta}}$$

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

A

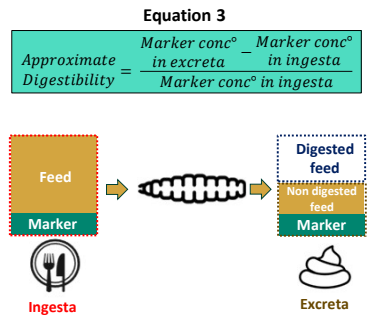
- **Method A:** measuring **Estimated Digestibility (ED; Eq. 2)**, calculated through a **mass balance between distributed feed and residual substrate (frass)**.
- ED of dry mass (DM) was measured at increasing larval densities (0 to 29 larvae/cm²), after a **fixed feeding time**. We hypothesized that **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.



Materials and methods

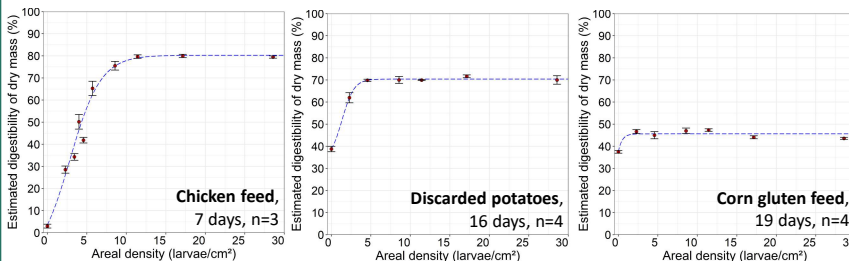
B

- **Method B:** addition of an **indigestible marker** (chromic oxide, Cr₂O₃) 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% Cr₂O₃ (%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.



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.

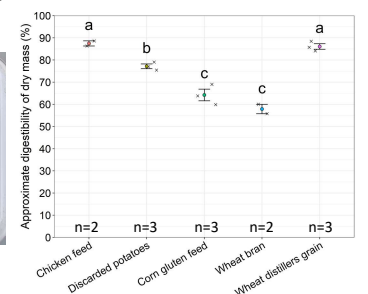
Results

B

- AD of DM determined with 1% Cr₂O₃ 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 Cr₂O₃)



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.

A

Conclusion

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).
 - 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.
- Weaknesses**

- Only considers digestion occurring in **larval gut**.
- Easily **repeatable** on different substrates.
- Cr₂O₃ 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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