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

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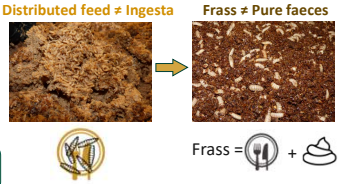
Background

- **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

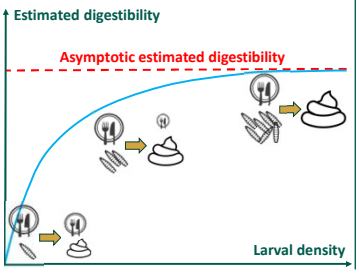
Materials and methods

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

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

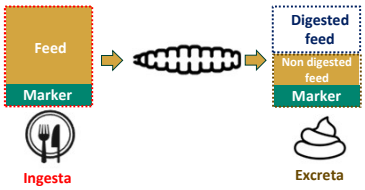


B

- **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

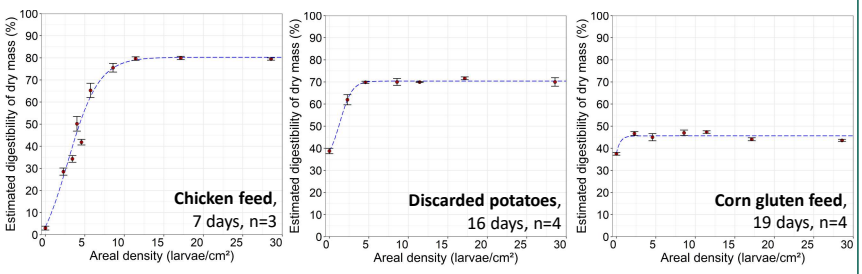
$$Approximate\ Digestibility = \frac{Marker\ conc^{o}\ in\ excreta - Marker\ conc^{o}\ in\ ingesta}{Marker\ conc^{o}\ in\ ingesta}$$



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].

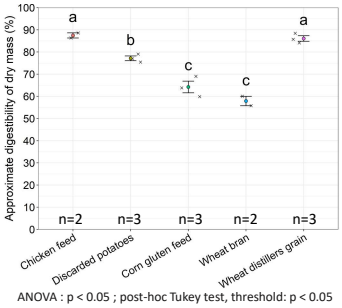


- In **discarded potatoes** after 7 days of feeding, **the frass was too moist and sticky** to properly separate it from the larvae. Feeding time was extended to **16 days** to allow proper evolution of frass texture. ED of DM in discarded potatoes increased with density but the asymptote was reached 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.

[1] Barragan-Fonseca *et al.*, 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. JIFF.
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[3] McGinnis & Kasting, 1964. Comparison of gravimetric and chromic oxide methods for measuring percentage utilization and consumption of food by phytophagous insects. J. of Ins. Phys.
[4] Guillaume *et al.*, 2023. Asymptotic estimated digestibility, a new indicator of black soldier fly (*Hermetia illucens*) conversion efficiency in relation to larval density. JIFF.
[5] Veldkamp, van Wikselaar & van Loon, personal communication.

08/2023