Circular economy and insect rearing on livestock residues: Challenge or opportunity?

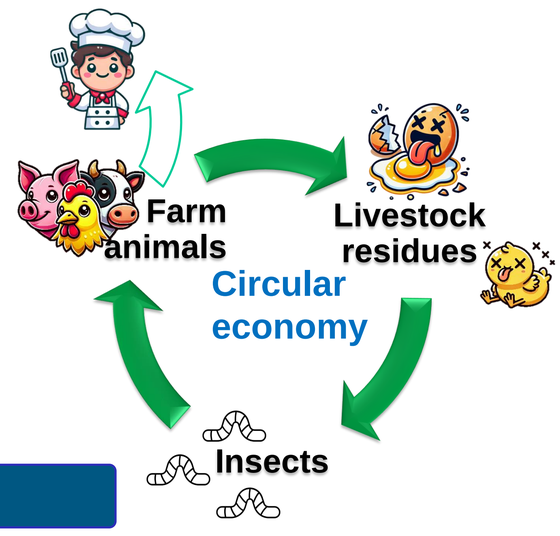
Marie-Caroline Lecrenier [1], Mariève Dallaire-Lamontagne [2], Abigaël Anselmo [1], Aline Marien[1], Marie-Hélène Deschamps & Vincent Baeten [1]
[1] Walloon agricultural Research Centre (CRA-W), Quality and Authentication of agricultural products Unit, Belgium;
[2] Université de Laval, Sciences animales, Faculté des sciences de l'agriculture et de l'alimentation, Canada.
Contact: m.lecrenier@cra.wallonie.be

Introduction & Objective

Insect meal has been identified as a promising alternative feed source. Recycling industrial and agricultural waste (e.g., former foodstuffs, slaughterhouse by-products, hatchery waste) as insect rearing substrates could support the Circular Economy action Plan of the EU Green Deal. However, such substrates are currently prohibited for several reasons, one of which is linked to the EU animal by-products regulation.

But is it **possible to control?**

The objectives of this study were to rear insects on a substrate containing fermented hatchery waste & to evaluate the insect meal by **LM, PCR & MS-proteomics** for detecting the presence of prohibited residues.



Materials & Methods

MATERIALS:

Livestock residues:
- ✓ Discarded eggs and embryos
- ✓ Carcasses of unmarketable chicks

} Lacto-fermented (7 days) [1]

Rearing substrate:
- ✓ Control diet (Gainesville)
- ✓ Fermented hatchery residues and chicken manure (25 % inclusion) diet

Insects:
- *Hermetia illucens*

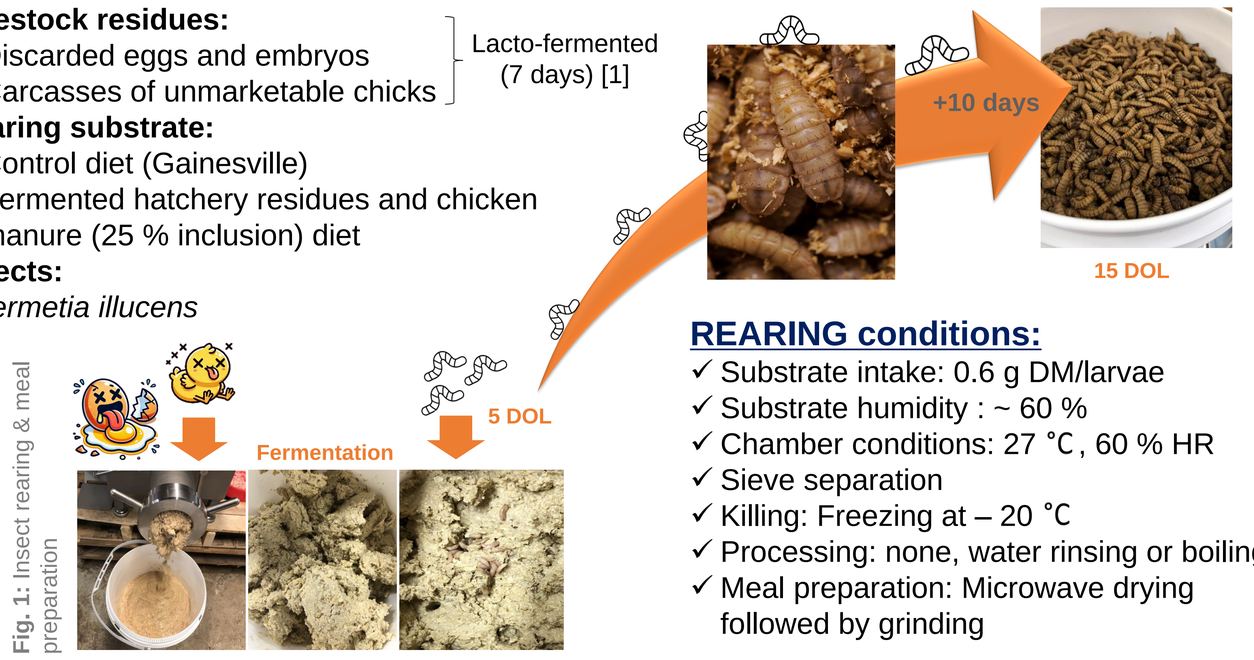


Fig. 1: Insect rearing & meal preparation

REARING conditions:
- ✓ Substrate intake: 0.6 g DM/larvae
- ✓ Substrate humidity : ~ 60 %
- ✓ Chamber conditions: 27 °C , 60 % HR
- ✓ Sieve separation
- ✓ Killing: Freezing at – 20 °C
- ✓ Processing: none, water rinsing or boiling
- ✓ Meal preparation: Microwave drying followed by grinding

Analytical METHODS:

MS-proteomics

Sample preparation protocol:
- ✓ Extraction: TRIS-urea buffer, pH 9.2
- ✓ Denaturation: DTT, IAA
- ✓ In-solution digestion: Trypsin
- ✓ Purification: tC18 SPE (Waters)

UHPLC-MS/MS:
- ✓ Acquity system (Waters)
- ✓ BEH C18 Column; 1.7 µm; 2.1 x 100 mm (Waters)
- ✓ Xevo TQ-XS triple quadrupole (Waters)

Targeted proteins (markers identified in previous studies [2-4]):
- ✓ RUMINANT: Casein, β-lactoglob., haemoglobin & collagen
- ✓ PIG: collagen
- ✓ POULTRY: collagen

LM & PCR:
- ✓ Following EURL-AP SOP (https://www.eurl.craw.eu/)

[1] M. Dallaire-Lamontagne, et *al.*, doi:10.1016/j.wasman.2024.01.010
[2] M.C. Lecrenier, et *al.*, doi: 10.1016/j.foodchem.2017.11.074.
[3] O. Fumiere, et *al.*, doi: 10.25518/1780-4507.20059.
[4] M.C. Lecrenier, et *al.*, doi.org/10.1021/acs.jafc.3c03253

Results & Discussion

1. Insect rearing

- ✓ Similar growth performance between diets
- ✓ Bioconversion rate on fermented hatchery residues and chicken manure diet : ~ 12 %

Sticky texture of the diet:
- ✓ Complexifies harvesting by sieving
- ✓ Leaves visible traces of residue on external larval surfaces (Fig. 2)

Fig. 2: Feed residues remain stuck on insects

2. Light microscopy (LM), PCR & MS-proteomics

As summarized in Table 1, Although **LM** did not reveal any bone or eggshell particles, **feathers** (Fig. 3) were observed in the flotate of all meals (no rinsing, cold rinsing, or boiling).

Table. 1: LM results

Sedimental fraction		Animal particles – Washing process: None	Rinsed	Boiled
Flotate		Feathers	Feathers	Feathers
Sediment	Terrestrial bones	< LOD	-	-
	Fish bones	-	-	-

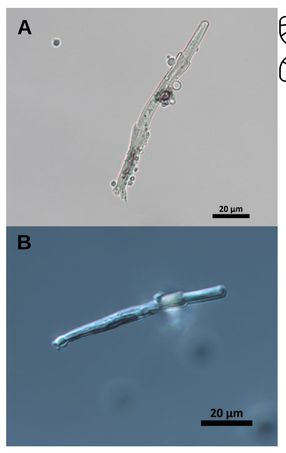


Fig. 3: Micrographs of feather particles (A = Brightfield, B = DIC)

PCR confirmed the presence of **poultry DNA** in all samples, with similar Ct values (Table 2). Samples are considered negative in **ruminant DNA** however traces were detected (Ct value > cut-off).

Table. 2: PCR results

DNA origin	Cut-off	Ct of amplification curves – Washing process: None	Rinsed	Boiled
Ruminant	36.70	Traces	Traces	Traces
Pig	40.03	-	-	-
Poultry	37.15	**33.5**	**33.7**	**32.7**

Table. 3: MS-Proteomics results (response= peak area ratio for the most intense MRM transition of the peptide marker and its ISTD)

Targeted origin	Proteins	Peptides	Response – Washing process: None	Rinsed	Boiled
Ruminant	Casein	FFVAPFPEVFGK	-	-	-
		HQGLPQEVLNENLLR	-	-	-
		NAVPITPTLNR	-	-	-
	β-lactoglobulin	LSFNPTQLEEQCHI	-	-	-
		VLVLDTDYK	0.163	0.128	0.240
	Haemoglobin α & β-chain	AAVTAFWGK	-	-	-
		EFTPVLQADFQK	-	-	-
		VGGHAAEYGAEALER	-	-	-
		VVAGVANALAHR	-	-	-
	Collagen I α-2 chain	GSTGEIGPAGPpGpPGLR	-	-	-
		GPpGESGAAGPTGPIGSR	-	-	-
		IGQpGAVGPAGIR	-	-	-
Pig	Collagen I α-2 chain	GFpGSpGNVGPAGK	-	-	-
		GIpGEFGLpGPAGPR	-	-	-
Poultry	Collagen I α-2 chain	GNVGLAGPR	**0.885**	**0.414**	**0.285**
		GLHGEFGVpGPAGPR	**0.036**	**0.023**	**0.016**
		GLVGEpGPAGAK	**0.321**	**0.170**	**0.128**
		GEIGPAGNVGPTGPAGPR	**0.002**	**0.002**	-

As shown in Table 3, **MS-Proteomics** yielded positive results for the detection of **poultry collagen**. However, a decrease in signal intensity was observed with the applied treatments.
One **β-lactoglobulin peptide** was also detected in all 3 samples with similar intensity. By applying the threshold of "at least 2 peptides detected per targeted protein", the samples are declared as negative for milk. However, these results tend to **explain the origin of the traces of ruminant DNA detected by PCR** and link it to an authorized by-product, probably coming from the lacto-fermentation agent.

Conclusion

After the insect rearing process, insects are separated from the feed substrate, but residual feed materials remained stuck on the larvae. The presence of these residues could be linked to safety risks.

These results underscore the importance of robust analytical methods to detect residual animal proteins in insect products. It offers data on cross-contamination risks and the effectiveness of the proposed analytical approaches.