Detection of downgraded fast food in insect feed by UHPLC-MS/MS proteomics

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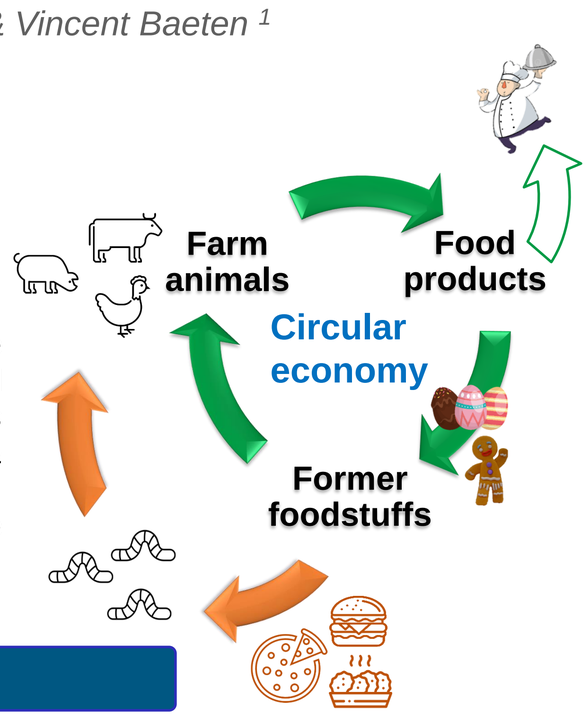
Introduction & Objective

As part of the European Green Deal, the European Commission promotes the reuse of **food waste** in animal feed. The EU's goal is to reduce dependence on critical feed materials like soya & also to reduce the amount of food waste.

Following the same sustainability strategy, the EU promotes the use of former foodstuffs (FFS) in feed, also in insect substrates. However, those **containing meat or fish remain prohibited.**
Is it possible to control?

At the end of rearing, insects are separated from the substrate but **residual feed materials** may still be present. In this study, insect meals were produced & evaluated by
MS-proteomics & PCR to detect the presence of meat residues.



Materials & Methods

MATERIALS:

Reference meats for markers selection:
- Raw + cooked meat
- Industrial meat: beef burger, ham and nuggets)

Substrates:
Poultry feed (Hen) + 10 % (w/w)
- Cheese burger 100 % beef
- Pizza “special” with ham & pepperoni
- Chicken nuggets

Insects:
- *Hermetia illucens,* 10-day-old larvae

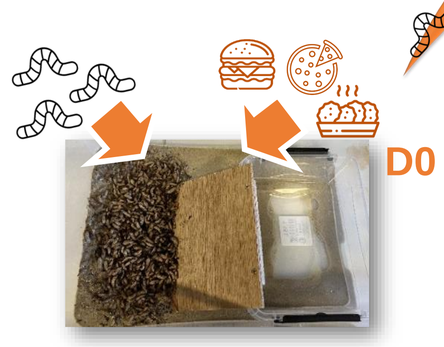


Fig. 1: Insect rearing & meal preparation

REARING conditions:
- Larvae/substrate proportion: 1/4
- Substrate humidity : ~ 60 %
- Chamber conditions: 24°C
- Separation by rinsing on sieve
- 24-hour fast (feed restriction)
- Killing: Freezing at - 20°C, 2 days
- Meal preparation:
  - Drying at 40°C, 3 days
  - + grinding

Analytical METHODS:

MS-proteomics:
Sample preparation protocol:
✓ Extraction: TRIS-urea buffer, pH 9.2
✓ Heat treatment: 95°C, 5 min
✓ 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 [1-3]):
✓ RUMINANT: Casein, β-lactoglob., haemoglobin & collagen
✓ PIG: collagen
✓ POULTRY: collagen

PCR:
✓ EURL-AP Standard Operating Procedures for the detection of RUMINANT, PIG & POULTRY DNA (https://www.eurl.craw.eu/)

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

Results & Discussion

1. Selection of the peptide markers on reference meats (MS-proteomics)

			Mass spec (Peak area)								
			Beef			Pork			Chicken		
Targeted origin	Proteins	Peptides	Raw meat	Cooked meat	Beef burger*	Raw meat	Cooked meat	Ham	Raw meat	Cooked meat	Nuggets*
Ruminant	Casein	FFVAPFPEVFGK	-	-	5.3E+04	-	-	-	-	-	3.9E+03
	β-lactoglobulin	LSFNPTQLEEQCHI	-	-	-	-	-	-	-	-	-
	Haemoglobin α & β-chain	AAVTAFWGK	1.7E+06	9.3E+05	1.0E+05	-	-	-	-	-	-
		EFTPVLQADFQK	1.1E+06	1.1E+06	1.2E+05	-	-	-	-	-	-
		VGGHAAEYGAEALER	9.4E+05	4.7E+05	1.4E+05	-	-	-	-	-	-
		VVAGVANALAHR	1.7E+07	1.0E+06	9.5E+05	-	-	-	-	-	-
	Collagen I α-2 chain	GEPGPAGAVGPAGAVGPR	-	2.3E+05	1.1E+06	-	-	-	-	-	-
		GSTGEIGPAGPpGPpGLR	-	9.1E+04	2.9E+05	-	-	-	-	-	-
		GPpGESGAAGPTGPIGSR	-	4.6E+04	1.7E+05	-	-	-	-	-	-
Pig	Collagen I α-2 chain	GFpGSpGNVGPAGK	-	-	-	-	1.1E+05	2.4E+05	-	-	-
		GIpGEFGLpGPAGPR	-	-	-	1.5E+03	5.9E+05	1.4E+06	-	-	-
Poultry	Collagen I α-2 chain	GNVGLAGPR	-	-	-	-	-	-	-	1.4E+05	1.8E+05
		GLHGEFGVpGPAGPR	-	-	-	-	-	-	-	1.4E+05	1.9E+05
		GLVGEpGPAGAK	-	-	-	-	-	-	-	1.2E+05	9.2E+04
		GEIGPAGNVGPTGPAGPR	-	-	-	-	-	-	-	6.9E+03	2.0E+04

Table 1: Results for UHPLC-MS/MS analyses reference meats

By MS-proteomics
- **Ruminant haemoglobin peptides:** detected in all beef meats (raw, cooked and industrial burger)
- **Collagen peptides:** detected in their respective meats, except in raw meats
- **Milk proteins:** detected in some industrial products. According to the labelling, these products may contain traces of milk.

for cooked & industrial meat

2. Evaluation of substrates and insect meals (MS-proteomics & PCR) at the beginning (D0) & at the end of the trial (D10)

Substrates:

By MS-proteomics
- D0: All peptides are detected in respective meats.
- D10: Meats are still detected (by **collagen peptides**) but peak area are decreased.

  Ruminant haemoglobin peptides, some ruminant & all poultry collagen peptides are no longer detected.

By PCR
- Respective DNA are detected on D0 & D10, but with an increase of the Ct value.

			Mass spec (Peak area)											
			Blank			Cheese burger			Pizza			Nuggets		
			Substrate		Insect meal	Substrate		Insect meal	Substrate		Insect meal	Substrate		Insect meal
Targeted origin	Proteins	Peptides	D0	D10	D10	D0	D10	D10	D0	D10	D10	D0	D10	D10
Ruminant	Casein	FFVAPF[…]	-	-	-	1.3E+06	-	-	2.8E+03	-	-	-	-	-
	β-lactoglobulin	LSFNPT[…]	-	-	-	1.4E+04	1.3E+03	-	9.9E+04	1.9E+04	-	-	-	-
	Haemoglobin α & β-chain	AAVTAF[…]	-	-	-	4.1E+03	-	-	-	-	-	-		-
		EFTPVL[…]	-	-	-	4.8E+03	-	-	-	-	-	-	-	-
		VGGHAA[…]	-	-	-	4.7E+03	-	-	-	-	-	-	-	-
		VVAGVA[…]	-	-	-	4.8E+04	-	-	-	-	-	-	-	-
	Collagen I α-2 chain	GEPGPA[…]	-	-	-	4.6E+04	-	-	-	-	-	-	-	-
		GSTGEI[…]	-	-	-	1.4E+04	8.0E+03	1.0E+03	-	-	-	-	-	-
		GPpGES[…]	-	-	-	1.3E+04	1.1E+04	1.3E+03	-	-	-	-	-	-
Pig	Collagen I α-2 chain	GFpGSp[…]	-	-	-	-	-	-	5.3E+04	1.7E+04	-	-	-	-
		GIpGEF[…]	-	-	-	-	-	-	1.8E+05	1.8E+04	7.0E+03		-	-
Poultry	Collagen I α-2 chain	GNVGLA[…]	-	-	-	-	-	-	-	-	-	2.0E+05	-	-
		GLHGEF[…]	-	-	-	-	-	-	-	-	-	9.2E+04	-	-
		GLVGEp[…]	-	-	-	-	-	-	-	-	-	8.4E+04	3.3E+04	-
		GEIGPA[…]	-	-	-	-	-	-	-	-	-	1.2E+04	-	-

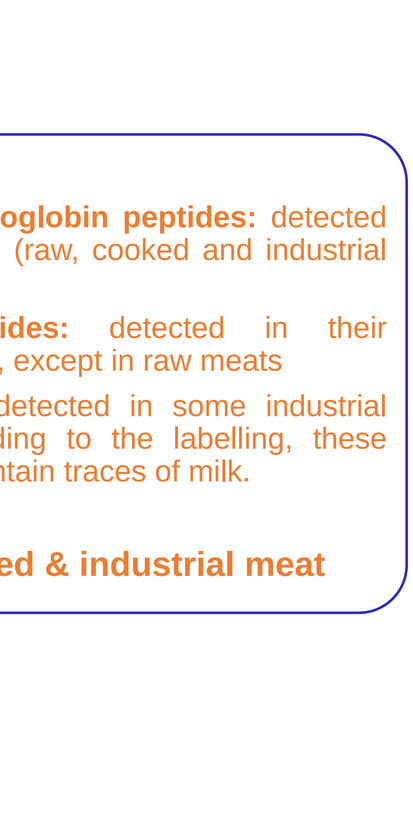


Fig. 2: Chromatograms of the 2 most intense transitions of ruminant and pig collagen peptides

Insect meals:

By MS-proteomics
- **Ruminant & Pig collagen peptides** are detected in insect meals produced on cheeseburgers & pizza, but poultry peptides are no longer detected in case of nuggets**.**

By PCR
- Respective DNA are also detected in insect meals.

		PCR (Ct)											
		Blank			Cheese burger			Pizza			Nuggets		
		Substrate		Insect meal	Substrate		Insect meal	Substrate		Insect meal	Substrate		Insect meal
Targeted origin	Cut-off	D0	D10	D10	D0	D10	D10	D0	D10	D10	D0	D10	D10
Ruminant	36.99	-	-	-	23.3	28.1	34.2	30.2	32.5	-	-	-	-
Pig	39.05	-	-	-	-	-	-	27.6	26.9	32.4	-	-	-
Poultry	37.54	-	-	-	-	-	-	-	-	-	25.5	27	31.2

Table 2 & 3 : Results for UHPLC-MS/MS & PCR analyses on substrates (D0 & D10) & insect meals (D10)

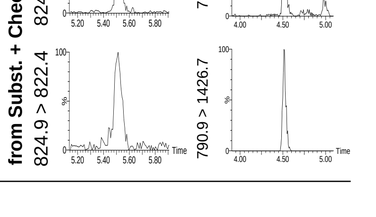


Fig. 3: Feed residues remain stuck on insects

Conclusion

Meat residues were detected in insect meals by MS-proteomics & PCR. Degradation of proteins are not the same for all proteins (haemoglobin *vs* collagen). **Collagen seems to be a good marker for the monitoring of the use of prohibited products.**
Other rearing conditions (washing method, fasting time, killing method,..) have to be tested to confirm this conclusion.