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Proteomic evaluation of the barrier role of insects for the indirect recycling of fast food in feed?

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Indirect recycling?

Recycling? Why?

- Reduction of dependency to soya
- Reduction of food waste
- Positive environmental & economic impact

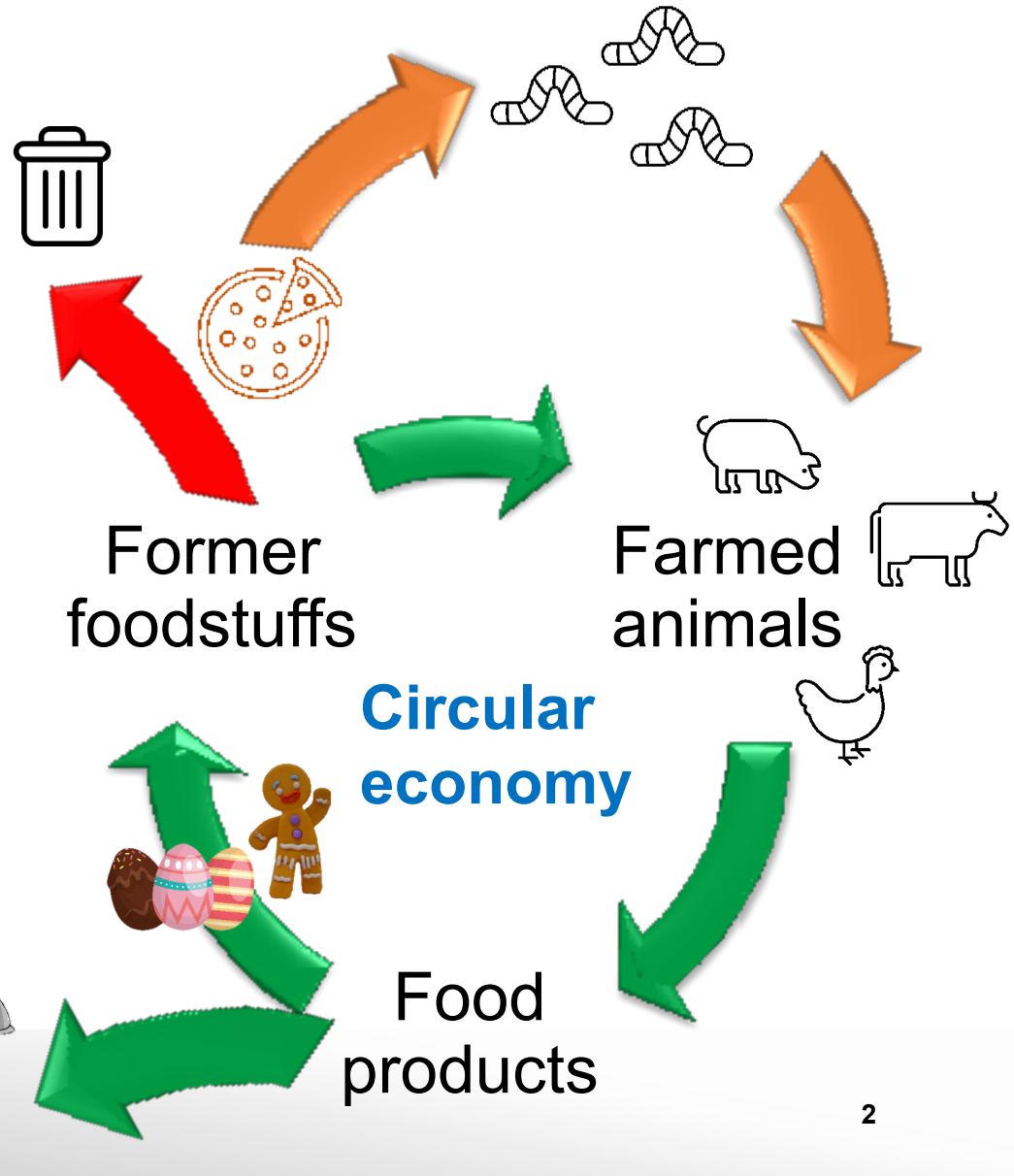
⇒ Former foodstuffs (FFS) are valuable feed materials

BUT

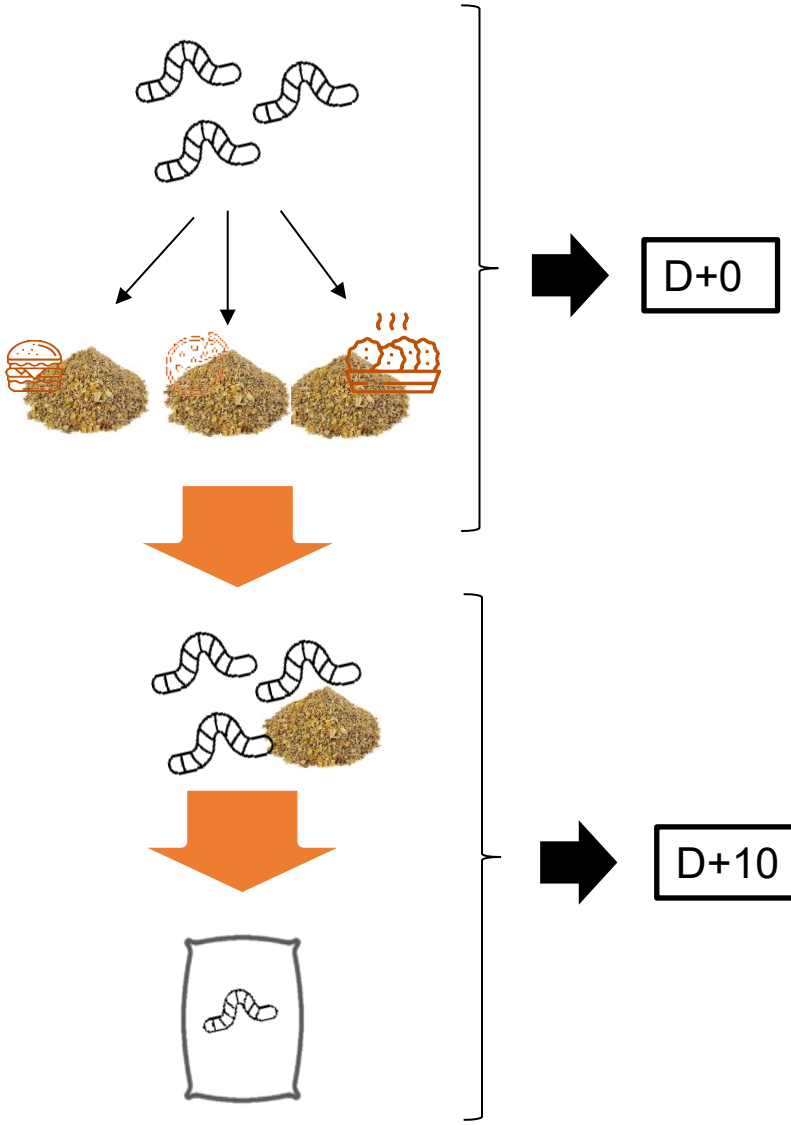
⇒ No meat and fish in farmed animal feed
(Regulation (EU) No 142/2011)

Indirect recycling through insect?

- Possible use of insect for feed conversion? Safety impact?
- Currently not authorised: reared insect = farmed animals
(Regulation (EC) No 1069/2009)



Objectives of the study



1. Selection of the peptide markers for animal protein detection (MS-proteomics)
2. Rearing insects on adulterated substrates and preparation of insect meal
3. ➡ Evaluation by MS-proteomics and PCR of substrate and insect meal (D+0 & D+10)

Barrier role?

Materials

Reference meat (for MS-proteomics development) :

- Raw meat
- Cooked meat
- Industrial meat

Substrates:

- Poultry feed (Hen):
  - No materials of animal origin
- Fast food items:
  - Cheese burger 100 % beef
  - Pizza “special” with ham & pepperoni
  - Chicken nuggets

3 Substrates =
Poultry feed + 10 % (w/w) Fastfood

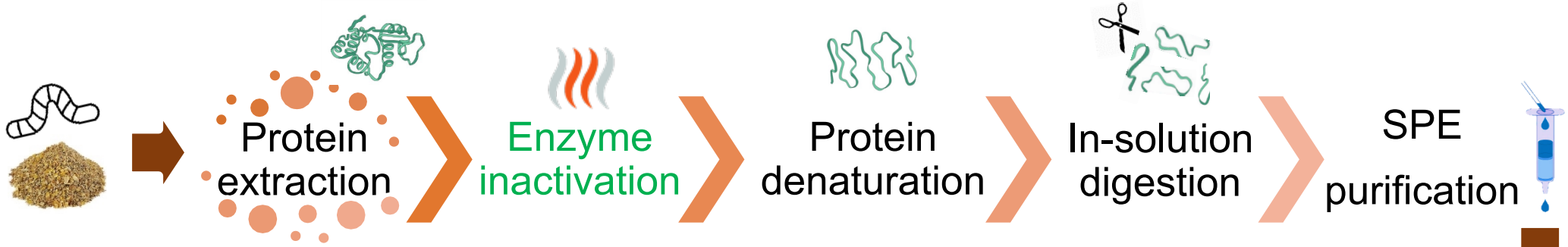
~ 1.6 % beef meat
~ 1.5 % pork meat
~ 5 % chicken meat

Insects:

- *Hermetia illucens*: larvae of 10 days

Analytical methods

1. MS-Proteomics:

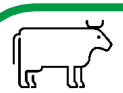


2. PCR:

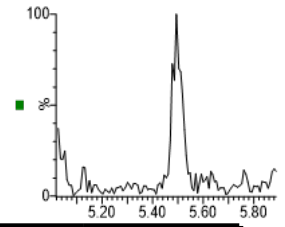
- According to the EURL-AP Standard Operating Procedures (https://www.eurl.craw.eu/)
- 3 Targets: Ruminant, Porcine & Poultry DNA

UHPLC-Triple quadrupole
Acquity-Xevo TQ-XS (Waters)

1. Selection of the peptide markers

Targeted origin	Targeted products	Proteins	Peptides	Precursor ion : *m/z* ^{z}	Transitions : *m/z* (fragment^{z})
	Milk products	Casein	FFVAPFPEVFGK	692.9 $^{2+}$	**920.5 ($y8^{+}$)** 991.5 ($y9^{+}$) 676.3 ($y6^{+}$)
		β-lactoglobulin	LSFNPTQLEEQCHI	858.4 $^{2+}$	**1254.6 ($y10^{+}$)** 928.4 ($y7^{+}$) 627.8 ($y10^{2+}$)
 **Ruminant**	Bone, skin, tendon, connective tissues,…	Collagen I α-2 chain	GEPGPAGAVGPAGAVGPR	758.9 $^{2+}$	**665.9 ($y16^{2+}$)** 781.4 ($y9^{+}$) 880.5 (10^{+})
			GSTGEIGPAGPpGPpGLR	824.9 $^{2+}$	**1047.6 ($y11^{+}$)** 822.4 ($y8^{+}$) 879.5 ($y9^{+}$)
			GPpGESGAAGPTGPIGSR	790.9 $^{2+}$	**841.5 ($y9^{+}$)** 1426.7 ($y16^{+}$) 912.5 ($y10^{+}$)
 **Pig**	Bone, skin, tendon, connective tissues,…	Collagen I α-2 chain	GFpGSpGNVGPAGK	637.3 $^{2+}$	**812.4 ($y9^{+}$)** 372.2 ($y4^{+}$) 1069.5 ($y12^{+}$)
			GIpGEFGLpGPAGPR	727.4 $^{2+}$	**667.3 ($y7^{+}$)** 837.5 ($y9^{+}$) 984.5 ($y10^{+}$)
 **Poultry**	Bone, skin, tendon, connective tissues,…	Collagen I α-2 chain	GNVGLAGPR	420.7 $^{2+}$	**570.3 ($y6^{+}$)** 513.3 ($y5^{+}$) 441.2 ($b5^{+}$)
			GLHGEFGVpGPAGPR	488.6 $^{3+}$	**667.3 ($y7^{+}$)** 797.4 ($b8^{+}$) 698.3 ($b7^{+}$)
			GLVGEpGPAGAK	534.8 $^{2+}$	**613.3 ($y7^{+}$)** 799.4 ($y9^{+}$) 456.2 ($b5^{+}$)
			GEIGPAGNVGPTGPAGPR	802.4 $^{2+}$	**809.4 ($y9^{+}$)** 624.3 ($y14^{2+}$) 752.4 ($y8^{+}$)

1. Selection of the peptide markers: Meat & substrates D+0



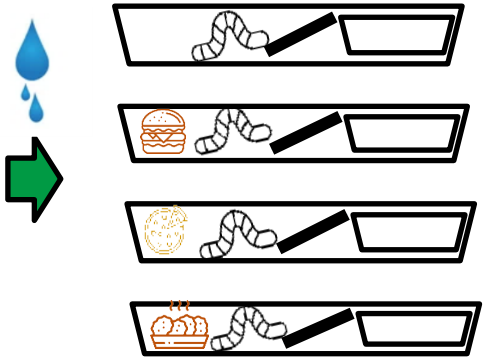
Feed materials:	Casein & β-lactoglob.		Collagen			Collagen		Collagen			
	FFV	LSF	GEP	GST	GPP	GFP	GIP	GNV	GLH	GLV	GEI
Raw meat	-	-	-	-	-	-	-	-	-	-	-
Cooked meat	-	-	2.3 10^5	9.1 10^4	4.6 10^4	-	-	-		-	-
Industrial Beef. burger*	5.3 10^4	-	1.1 10^6	2.9 10^5	1.7 10^5	-	-			-	-
Subst. + 10 % Cheese burger (D+0)	1.3 10^6	1.4 10^4	4.6 10^4	1.4 10^4	1.3 10^4	-	-			-	-
Raw meat	-	-	-	-	-	-	1.5 10^3			-	-
Cooked meat	-	-	-	-	-	1.1 10^5	5.9 10^5	-	-	-	-
Ham	-	-	-	-	-	2.4 10^5	1.4 10^6	-	-	-	-
Subst. + 10 % Pizza (D+0)	2.8 10^3	9.9 10^4	-	-	-	5.3 10^4	1.8 10^5	-	-	-	-
Raw meat	-	-	-	-	-	-	-	-	-	-	-
Cooked meat	-	-	-	-	-	-	-	1.4 10^5	1.4 10^5	1.2 10^5	6.9 10^3
Industrial Chicken burger*	3.9 10^3	-	-	-	-	-	-	1.8 10^5	1.9 10^5	9.2 10^4	2.0 10^4
Subst. + 10 % Nuggets (D+0)	-	-	-	-	-	-	-	2.0 10^5	9.2 10^4	8.4 10^4	1.2 10^4

*may contain traces of milk

2. Insect rearing and preparation of insect meal

+10 %
Fastfood

Grinding

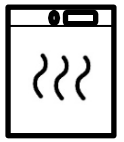


100 g Larvae/400 g substrate
+/- 60 % humidity

Climatic chamber
(24°C)
10 days

Rinsing
+ 24-hour fast
(feed restriction)

Freezing
(-20 °C)
2 days



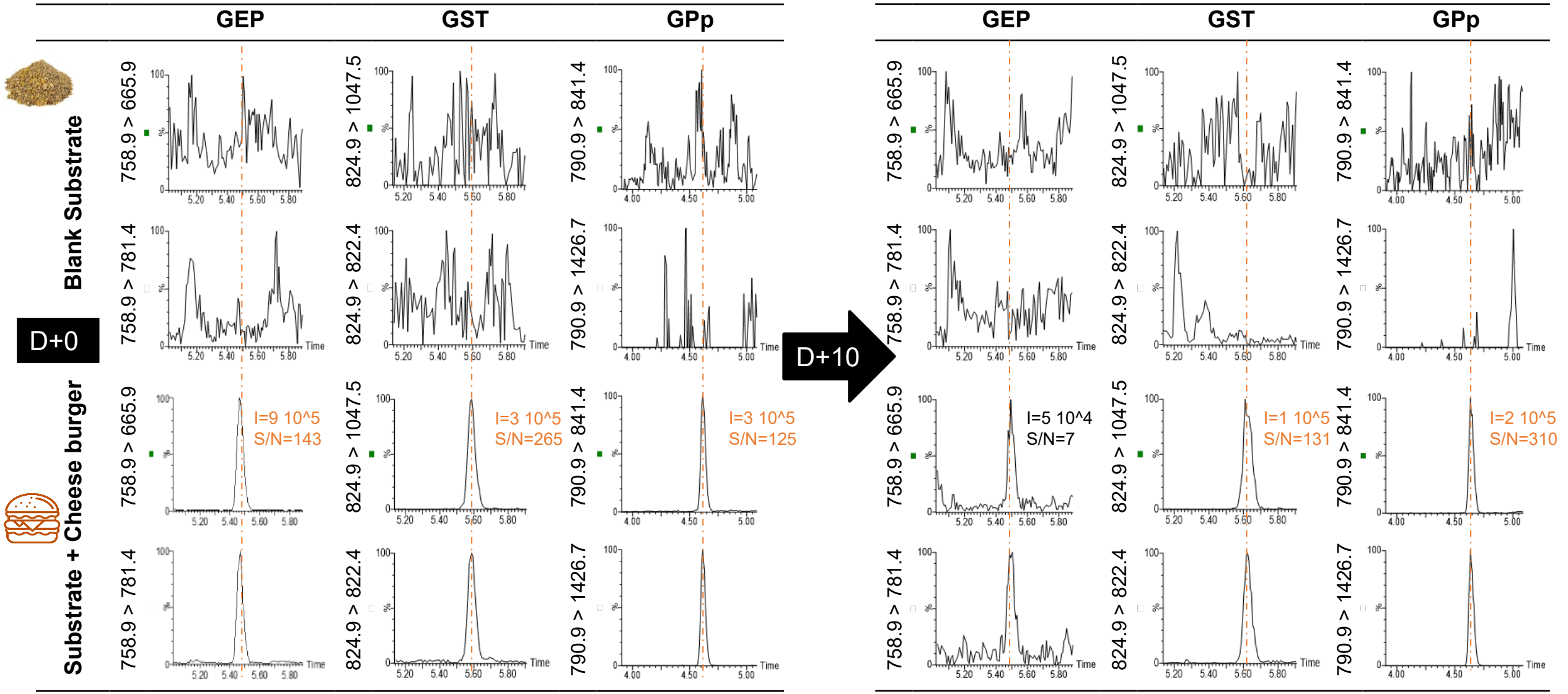
Drying
(40°C)
3 days

Grinding

3. Evaluation of substrates (D+0 & D+10) by Mass Spec and PCR

Mass Spec (peak area)												
		Casein & β-lactoglob.		Collagen			Collagen		Collagen			
Feed materials:		FFV	LSF	GEP	GST	GPP	GFP	GIP	GNV	GLH	GLV	GEI
Blank subst.	D0	-	-	-	-	-	-	-	-	-	-	-
	D10	-	-	-	-	-	-	-	-	-	-	-
+	D0	1.3 10^6	1.4 10^4	4.6 10^4	1.4 10^4	1.3 10^4	-	-	-	-	-	-
	D10	-	1.3 10^3	-	8.0 10^3	1.1 10^4	-	-	-	-	-	-
+	D0	2.8 10^3	9.9 10^4	-	-	-	5.3 10^4	1.8 10^5	-	-	-	-
	D10	-	1.9 10^4	-	-	-	1.7 10^4	1.8 10^4	-	-	-	-
+	D0	-	-	-	-	-	-	-	2.0 10^5	9.2 10^4	8.4 10^4	1.2 10^4
	D10	-	-	-	-	-	-	-	-	-	3.3 10^4	-

Results: Substrate with Cheese burger

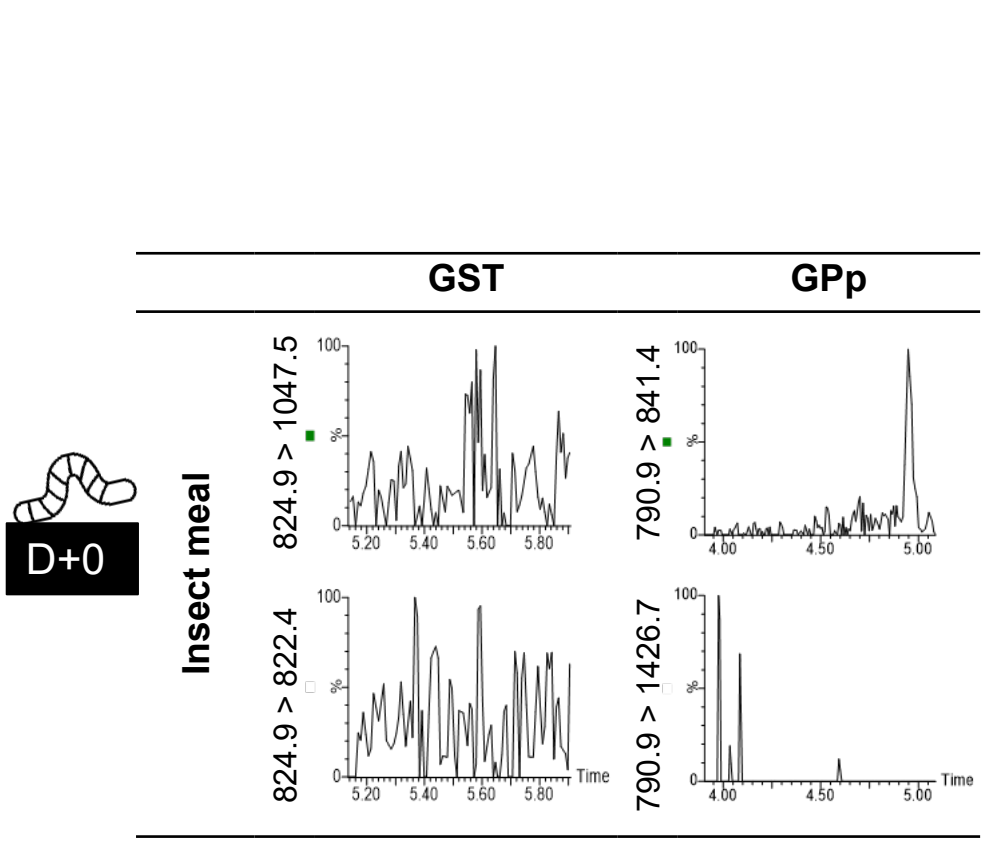


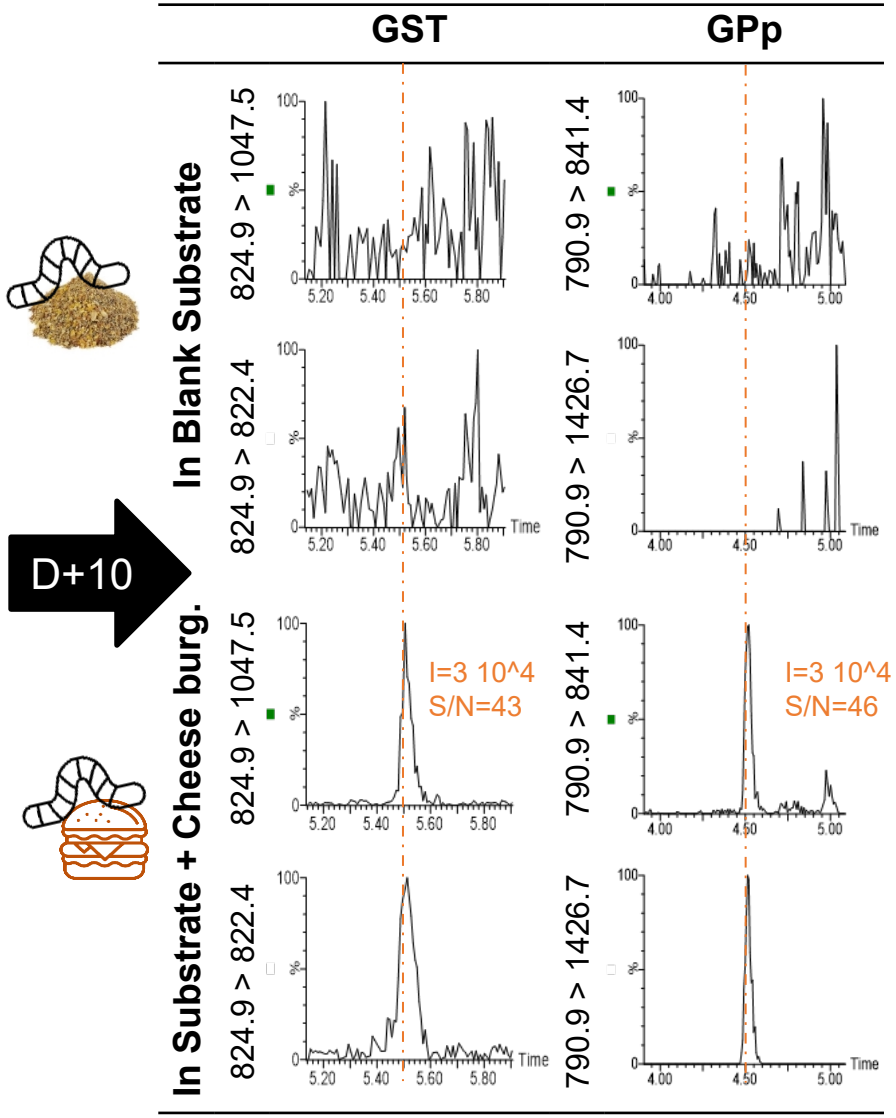
3. Evaluation of insect meal (D+0 & D+10) by Mass Spec and PCR

		Mass Spec (peak area)										PCR (Cut-off/Ct)		
		Casein & β-lactoglob.		Collagen			Collagen		Collagen					
Feed materials:		FFV	LSF	GEP	GST	GPP	GFP	GIP	GNV	GLH	GLV	GEI			
Blank subst.	D0	-	-	n/i	-	-	-	-	n/i	n/i	-	n/i	-	-	-
	D10	n/i	-	n/i	-	-	-	-	n/i	-	-	n/i	-	-	-
+	D10	n/i	n/i	n/i	1.0 10^3	1.3 10^3	-	-	-	-	-	n/i	34.2	-	-
+	D10	n/i	-	n/i	-	-	-	7.0 10^3	n/i	-	-	n/i	-	32.4	-
+	D10	n/i	-	n/i	-	-	-	-	n/i	-	-	n/i	-	-	31.2
											Cut-off:		36.99	39.05	37.54

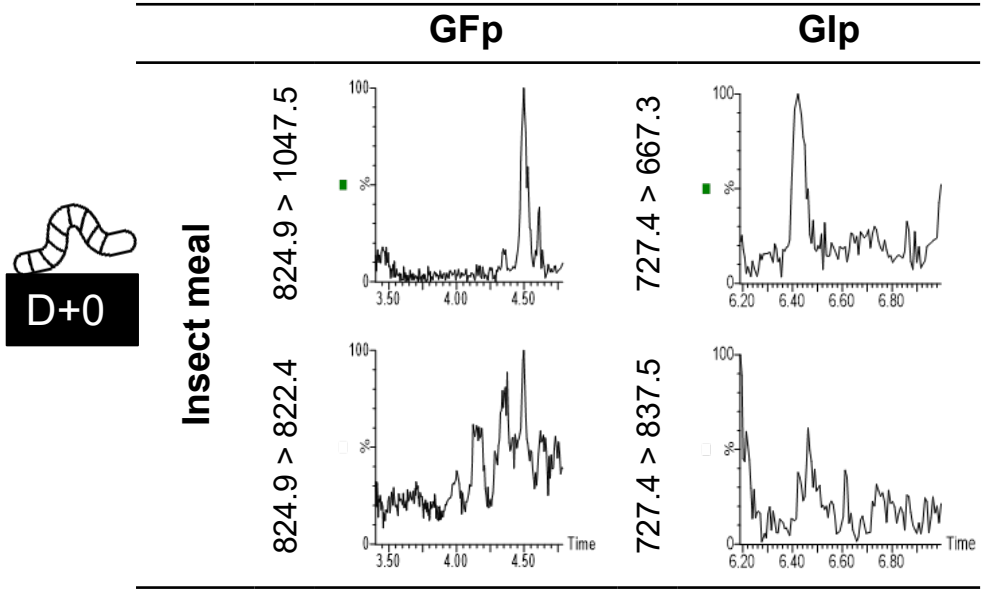
n/i = not interpretable

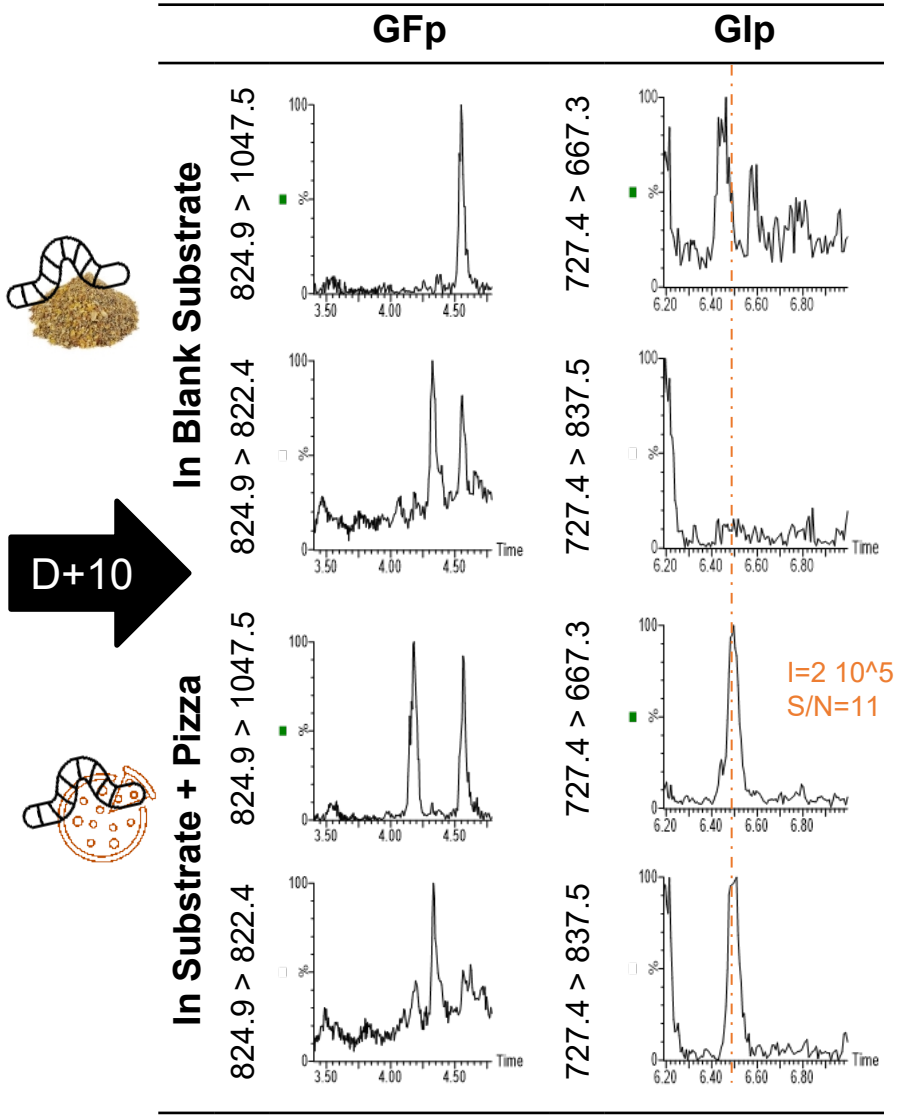
Results: Insect meal with Cheese burger





Results: Insect meal with Pizza





Discussion & Conclusion

Safety barrier role of insects ?

Solutions?

- Difficult to remove residual feed materials

⇒ other washing methods

- Other killing methods (boiling, …)

⇒ influence on analytical results

- 24-hour fasting time enough?

⇒ increase time

- Other insect species? (e.g. *Tenebrio molitor*)

⇒ Same results on dry substrate?

Thank you for your attention

Marvin Aerts

Master's thesis

UCLouvain