Chemical & microbiological safety of insect rearing on yet to be legally authorised residual streams

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SAFE INSECTS

Insects and residual streams

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- Black Soldier Fly *(Hermetia illucens)*
- Mealworm *(Tenebrio molitor)*

- Poultry manure
- Cat II meat meal (from animal rendering)
- Organic waste from household kitchens (GFE)
- Supermarket mix

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Aim of the project

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- Ensure safety (and technically feasability) for

  legally not permitted waste streams or residual flows

  used as substrate for insect rearing

  to be used for food or feed

  To enforce and substantiate needed legal changes in EU legislation

- Create value out of residual streams by using this as substrate for insect rearing

BSF experiment

Crates with 10 kg substrate

7-days-old larvae

Duration experiment: 1 week

1. Chicken feed

2. GFE

3. Broiler manure

4. Supermarketmix

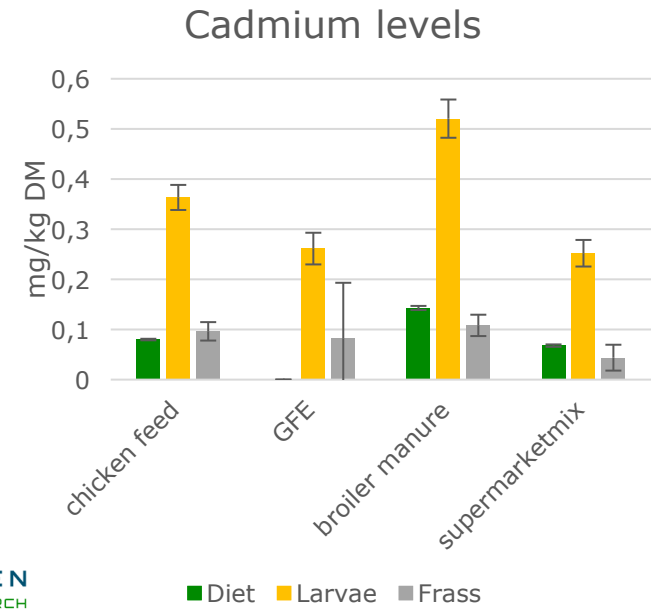
YMW experiment

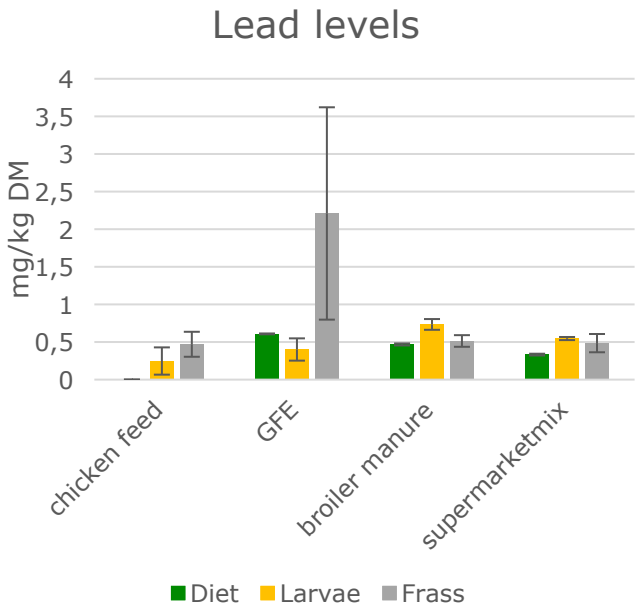
	Dry ingredient	Wet ingredient
Diet 1	Chicken feed	Carrot
Diet 2	Broiler manure	Carrot
Diet 3	Wheat	Carrot
Diet 4	Cat 2 meat meal	Carrot
Diet 5	Wheat	GFE
Diet 6	Wheat	Supermarketmix

Duration experiment: 20 days
15.800 YMW/crate

Heavy metals BSF

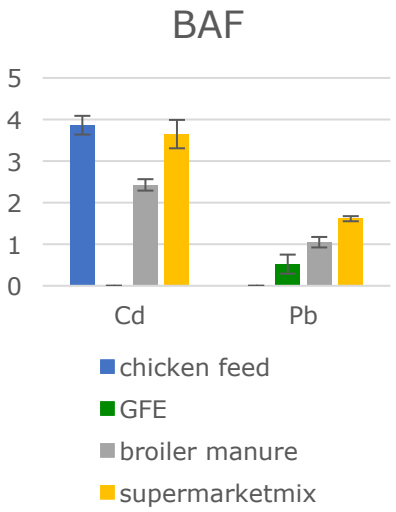
- No mercury or arsenic found in any diet
- Cd and Pb well below maximum levels for feed (ML Cd: 1 and Pb 10 mg/kg)

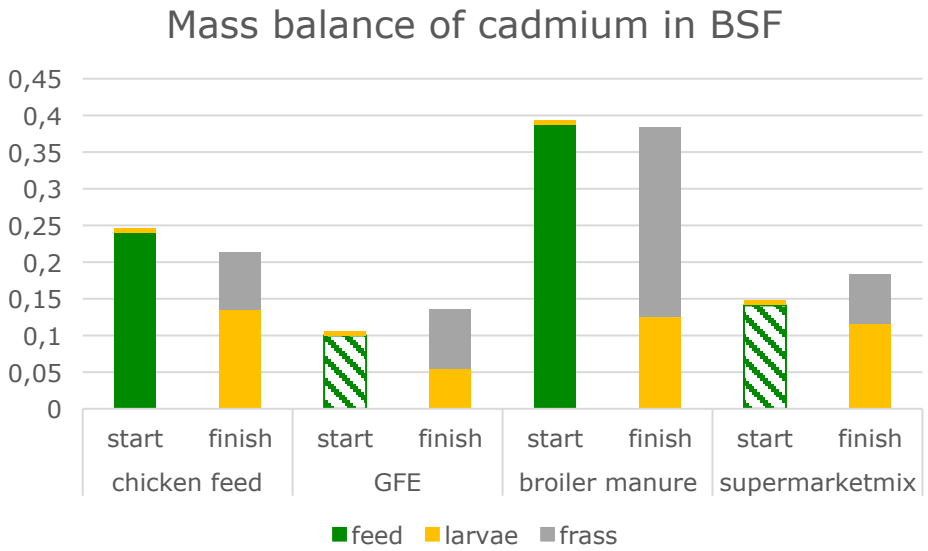




Heavy metals BSF

- Cd in BSF: bioaccumulation (BAF)

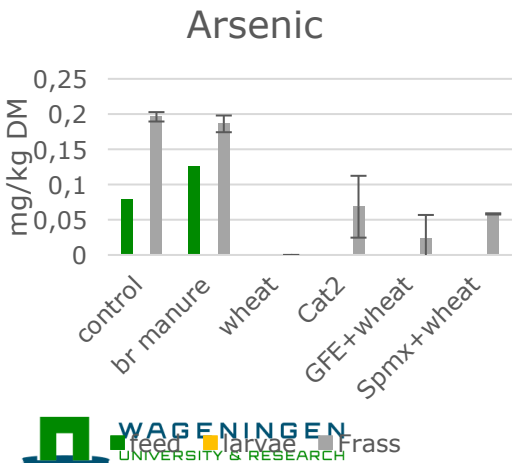


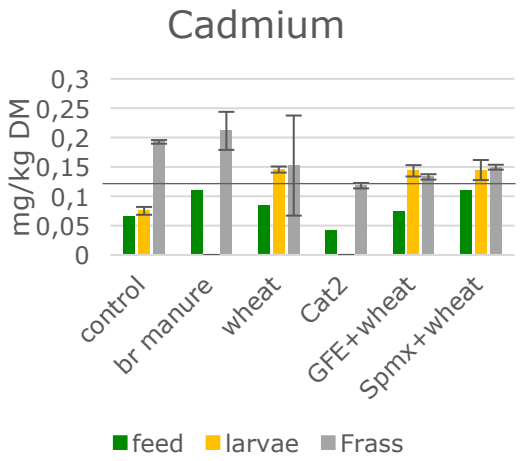


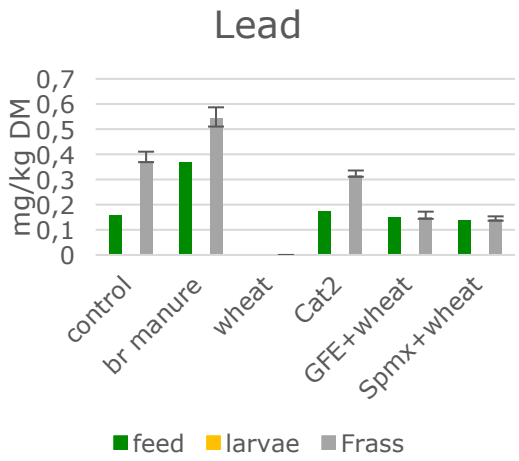
* LOQ 0,02 mg/kg, <loq assumed 0.5loq

Heavy metals YMW

- No mercury found in any diet, no lead and arsenic found in larvae
- Levels well below maximum levels for feed (ML As: 2, Cd: 1 and Pb 10 mg/kg)
- Cadmium in insects close to maximal levels for meat (ML Cd:0,05 wet basis)

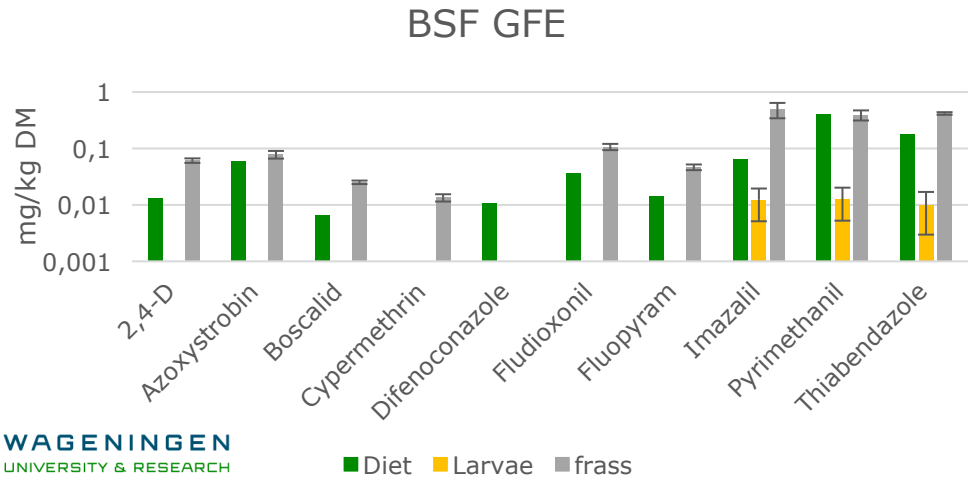


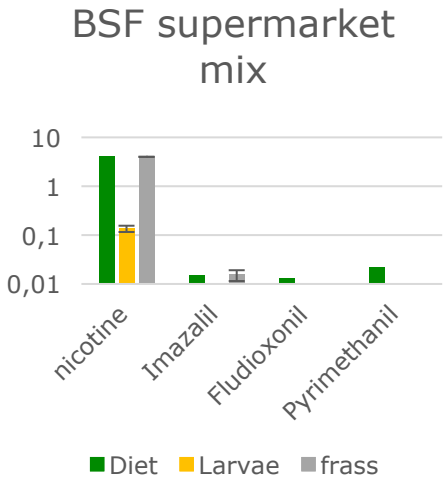




Pesticides BSF

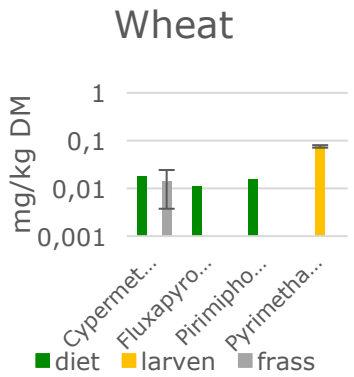
- Broad screening on pesticides (>250 substances). No pesticide residues found in control diet, broiler manure and t=0 larvae
- Few pesticides carried over to larvae
- Low concentrations in the larvae: no accumulation and a much lower concentration in larvae compared to diet

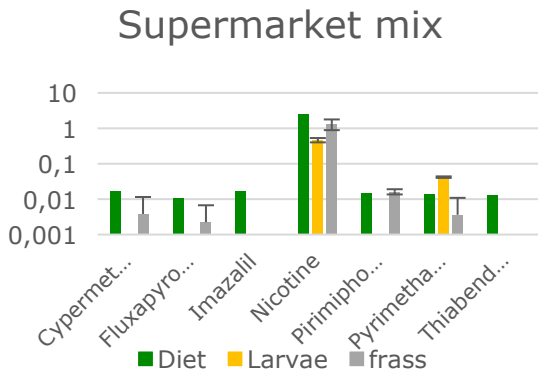


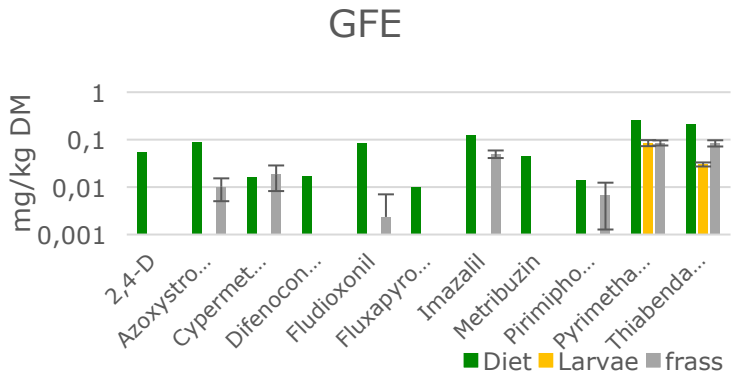


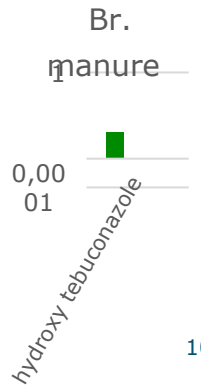
Pesticides YMW

- No pesticide residues found in chicken feed, Cat 2 meat meal, carrots and t=0 larvae
- Few pesticides carry over to larvae
- No accumulation of pesticides



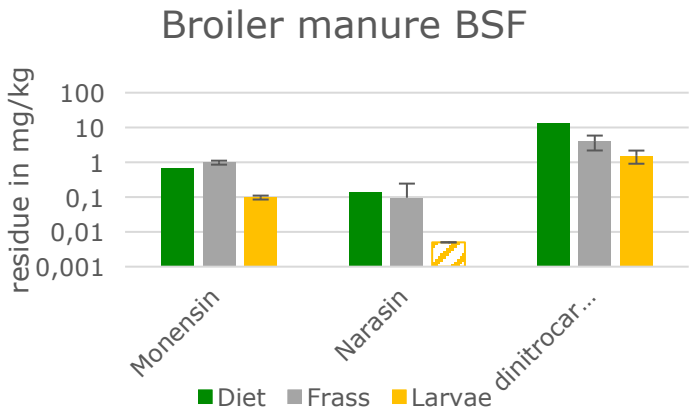


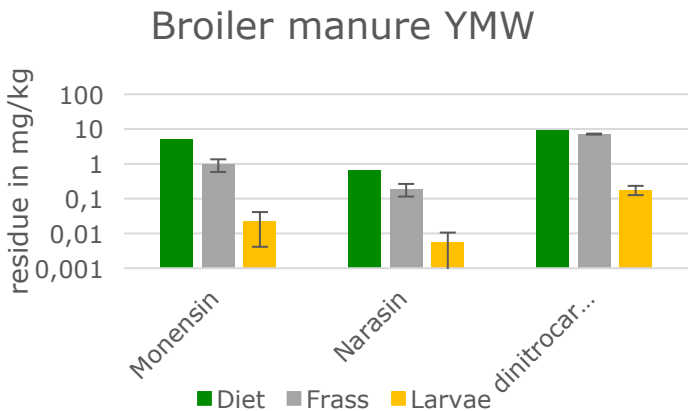




Veterinary drugs

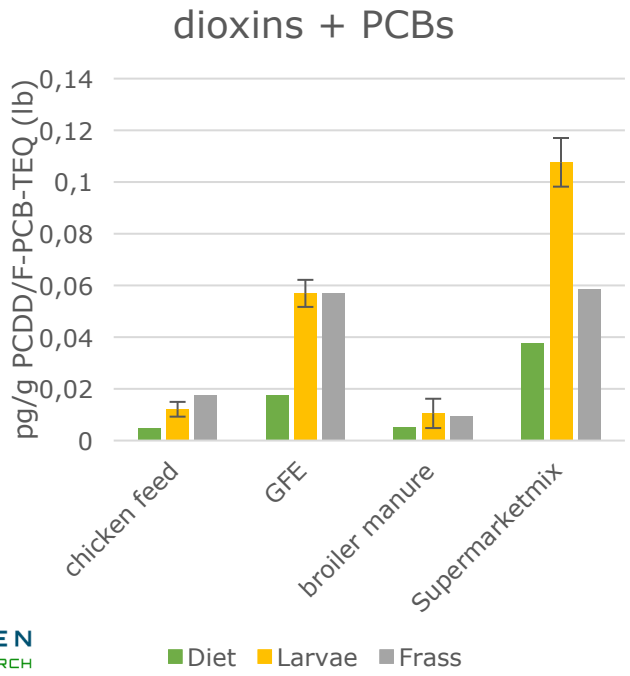
- 71 compounds analysed, incl antibiotics and coccidiostats
- 3 coccidiostats found in the broiler manure diets, larvae and frass
- Concentrations in BSFL 10-fold lower and in YMW 100-fold lower than in the diet

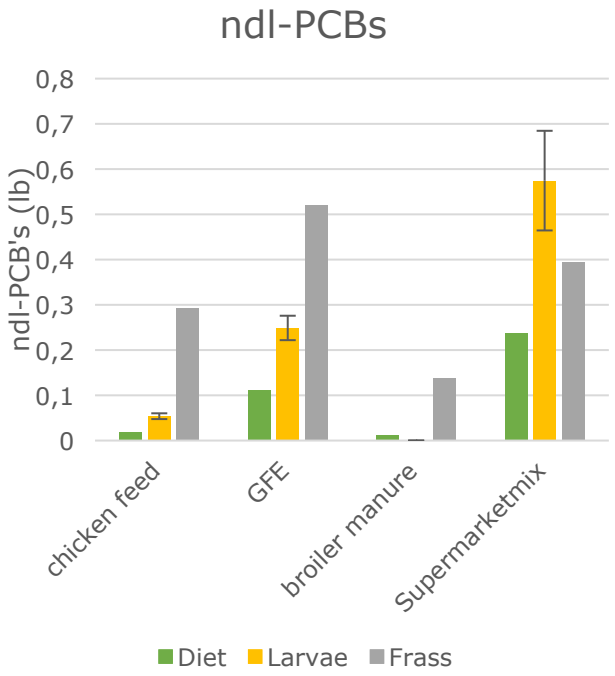




Dioxins and PCBs BSF

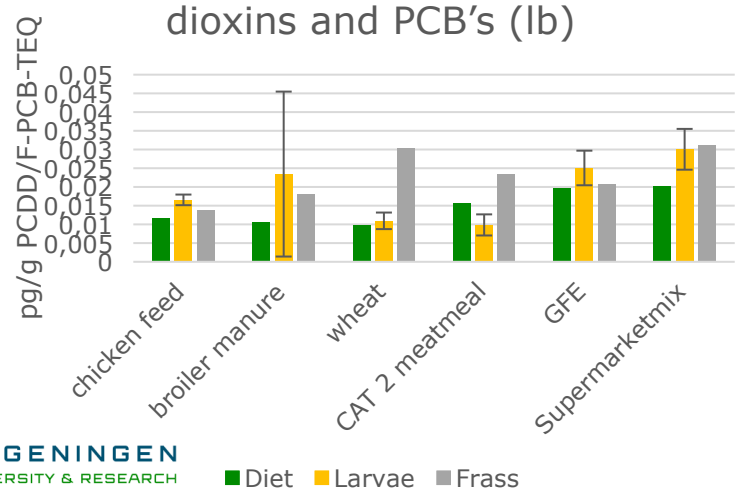
- Dioxins and PCB's levels well below maximum limits for feed
- ML TEQ = 1,5 pg/g, ndl PCB's = 10 ng/g

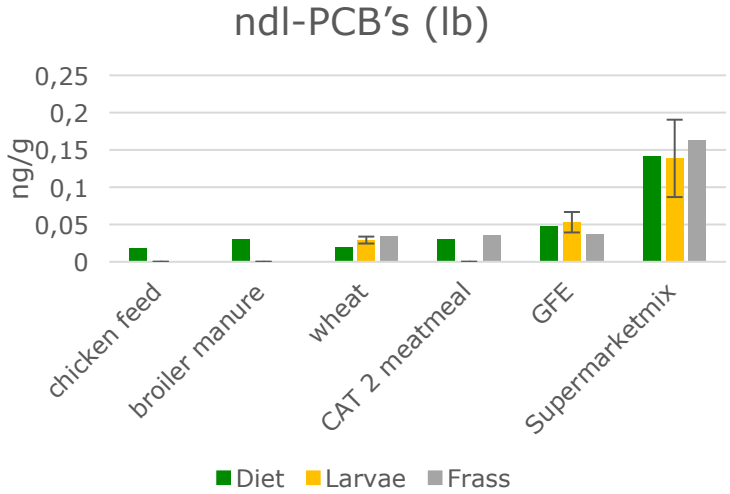




Dioxins and PCBs YMW

- Dioxins and PCB's levels well below maximum levels for feed (ML TEQ = 1,5 pg/g, ndl PCB's = 10 ng/g)
- Mealworms well below maximum limits for meat (e.g. pigs =1,25 pg/g fat)





Microbiological analysis BSF experiment

	Start	D1 control			D2 GFE		
	Larvae	Diet	Larvae	Residue	Diet	Larvae	Residue
Listeria monocytogenes	no	no	no	no	no	no	No
Bacillus cereus	<1.0E+2	<1.0E+2	<1.0E+2	<1.0E+2	>1.5E+5	2.6E+4	<1.0E+2
Clostridium perfringens	<1.0E+2	<1.0E+2	<1.0E+2	<1.0E+2	6.4E+3	<1.0E+2	6.6E+2
Campylobacter	NA	NA	NA	NA	NA	NA	NA
Salmonella	**A**	NA	**SEEP**	**SEEP**	NA	SEEP	SEEP
MRSA	NA	NA	NA	NA	NA	NA	NA

	D3 broiler manure			D4 supermarketmix			Legal
	Diet	Larvae	Residue	Diet	Larvae	Residue	Limit
Listeria monocytogenes	no	no	no	no	no	no	any
Bacillus cereus	<1.0E+2	<1.0E+2	<1.0E+2	3.8E+3	4.5E+3	<1.0E+2	1.0E+5
Clostridium perfringens	<1.0E+2	<1.0E+2	<1.0E+2	7.7E+2	2.1E+3	1.5E+4	1.0E+5
Campylobacter	NA	NA	NA	NA	NA	NA	
Salmonella				NA	**SEEP**	**SEEP**	
MRSA				NA	NA	NA	

Microbiological analysis YMW experiment

- *Campylobacter, Staphylococcus aureus (MRSA), Salmonella and Listeria monocytogenes were* not detected in substrate, larvae and frass
- *Bacillus cereus*: in GFE >1.5E5, in CATII 4.7E1 and in SUP 5.7E3

  in larvae and frass detected in levels <1E5
- *Clostridium perfringens*: in SMM 8.8E2

  in SMM larvae: <1.0E2 SMM frass: 4.5E1
- Total aerobic cell count: high values found

Overall conclusions

- Monitoring substrates on cadmium needed due to bioaccumulation
- Pesticides, veterinary drugs no bioaccumulation
- Dioxins and PCB levels found were well below legal limits
- Pathogens levels found in general lower in the larvae

Acknowledgements

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Wageningen Food Safety Research

Wageningen Bioveterinary Research

Wageningen Livestock Research

Wageningen Food Biobased Research

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nijsen/granico

DORSET GP DORSET GROUP

MEATCO BV

Nevedi

AVI NED

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DARLING INGREDIENTS

AMS AMSTERDAM INSTITUTE FOR ADVANCED METROPOLITAN SOLUTIONS

			Bacillus cereus		Aerobic bacteria		Clostridium perfringens		sulfite reducing Clostridia	
		diet	larvae	frass	larvae	frass	larvae	frass	larvae	frass
B1	**control**		<1.0E2	<1.0E2	1.8E8	>3.0E8	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B2	**broiler manure**		<1.0E2	<1.0E2	>2.1E8	>3.0E8	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B3	**wheat**		<1.0E2	<1.0E2	1.3E8	1.5E8	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B4	**cat 2 meat meal**		<1.0E2	<1.0E2	>2.9E8	>3.0E8	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B5	**GFE +wheat**		6.0E3	4.6E4	6.7E7	4.5E6	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B6	**GFE +meat meal**		2.2E4	4.9E4	1.2E8	7.6E6	<1.0E2	<1.0E2	<1.0E2	5.0E2
B7	**supermarketmix + wheat**		1.0E3	1.1E3	5.8E7	1.6E6	<1.0E2	4.5E1	<1.0E2	1.0E2
B8	**supermarketmix + meat meal**		1.6E3	5.5E2	>2.8E8	5.8E6	1.2E4	<1.0E2	1.3E5	1.6E3

Mealworm experiment – Microbiological hazards (D0)

1.0E+5 1.0E+5

	Listeria mono-cytogenes	*Bacillus cereus*	sulfite reducing Clostridia	*Clostridium perfringens*	Campylobacter	Staphylococcus aureus (MRSA)	*Salmonella*
YMW	Not detected	<1.0E2	<1.0E2	<1.0E2	Not detected	Not detected	Not detected

	Aerobic bacteria	*Aerobic spore count (HAS 1)*	*Aerobic spore count (HAS 2)*	Anaerobic spore count (HAS 1)	Anaerobic spore count (HAS 2)
YMW	7.6E7	1.4x10^2	1.4x10^2	Not detected	Not detected

5.0E+6

Mealworm experiment – Microbiological hazards (D0)

		1.0E+5		1.0E+5			
	Listeria mono-cytogenes	*Bacillus cereus*	sulfite reducing Clostridia	*Clostridium perfringens*	Campylobacter	Staphylococcus aureus (MRSA)	*Salmonella*
Carrot	Not detected	<1.0E2	<1.0E2	<1.0E2	Not detected	Not detected	Not detected
Standard feed	Not detected	<1.0E2	<1.0E2	<1.0E2	Not detected	Not detected	Not detected
BRM	Not detected	<1.0E2	<1.0E2	<1.0E2	Not detected	Not detected	Not detected
Wheat bran	Not detected	<1.0E2	<1.0E2	<1.0E2	Not detected	Not detected	Not detected
CATII	Not detected	4.7E1	<1.0E2	<1.0E2	Not detected	Not detected	Not detected
GFE	Not detected	>1.5E5	1.5E3	<1.0E2	Not detected	Not detected	Not detected
SMM	Not detected	5.7E3	4.0E3	8.8E2	Not detected	Not detected	Not detected

Mealworm experiment – Microbiological hazards (D0)

	Aerobic bacteria	*Aerobic spore count (HAS 1)*	*Aerobic spore count (HAS 2)*	Anaerobic spore count (HAS 1)	Anaerobic spore count (HAS 2)
Carrot	4.9E6	NA	NA	NA	NA
Standard feed	5.9E4	3.1x10^3	2.9x10^2	1.4x10^2	1.5x10^4
BRM	4.9E5	6.5x10^5	3.9x10^5	NA	4.0x10^2
Wheat bran	6.3E4	2.2x10^2	NA	1.5x10^5	NA
CATII	9.2E1	NA	5.4x10^3	NA	NA
GFE	1.4E7	1.8x10^6	1.06x10^6	1.9x10^3	2.1x10^5
SMM	1.4E5	2.6x10^4	5.2x10^3	4.4x10^3	1.23x10^3
	5.0E+6				

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Mealworm experiment – Microbiological hazards (D23)

		Bacillus cereus		Aerobic bacteria		Clostridium perfringens		sulfite reducing Clostridia	
		larvae	frass	larvae	frass	larvae	frass	larvae	frass
B1	**control**	<1.0E2	<1.0E2	1.8E8	>3.0E8	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B2	**broiler manure**	<1.0E2	<1.0E2	>2.1E8	>3.0E8	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B3	**wheat**	<1.0E2	<1.0E2	1.3E8	1.5E8	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B4	**cat 2 meat meal**	<1.0E2	<1.0E2	>2.9E8	>3.0E8	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B5	**GFE +wheat**	6.0E3	4.6E4	6.7E7	4.5E6	<1.0E2	<1.0E2	<1.0E2	<1.0E2
B6	**GFE +meat meal**	2.2E4	4.9E4	1.2E8	7.6E6	<1.0E2	<1.0E2	<1.0E2	5.0E2
B7	**supermarketmix + wheat**	1.0E3	1.1E3	5.8E7	1.6E6	<1.0E2	4.5E1	<1.0E2	1.0E2
B8	**supermarketmix + meat meal**	1.6E3	5.5E2	>2.8E8	5.8E6	1.2E4	<1.0E2	1.3E5	1.6E3

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1.0E+5 5.0E+6 1.0E+5

Mealworm experiment – Microbiological hazards (D23)

		Campylobacter		*Staphylococcus aureus (MRSA)*		*Salmonella*	
		larvae	frass	larvae	frass	larvae	frass
B1	**CF & carrot**	Not detected		Not detected		Not detected	
B2	**BRM & carrot**	Not detected		Not detected		Not detected	
B3	**Wheat Bran & carrot**	Not detected		Not detected		Not detected	
B4	**CAT II & carrot**	Not detected		Not detected		Not detected	
B5	**GFE & Wheat Bran**	Not detected		Not detected		Not detected	
B6	**GFE & CAT II**	Not detected		Not detected		Not detected	
B7	**SMM & Wheat Bran**	Not detected		Not detected		Not detected	
B8	**SMM & CAT II**	Not detected		Not detected		Not detected	

Mealworm experiment – Microbiological hazards (D23)

		Aerobic spore count		*Anaerobic spore count*	
		larvae	frass	larvae	frass
B1	**CF & carrot**	Not detected		Not detected	
B2	**BRM & carrot**	5x10^4		Not detected	
B3	**Wheat Bran & carrot**	NA		Not detected	
B4	**CAT II & carrot**	NA		Not detected	
B5	**GFE & Wheat Bran**	2,6x10^5		1,4x10^4	
B6	**GFE & CAT II**	1,3x10^5		1,11x10^4	
B7	**SMM & Wheat Bran**	2,35x10^3		Not detected	
B8	**SMM & CAT II**	2,2x10^3		Not detected	

BSF experiment – Microbiological hazards (D0)

	Start	D1 | CF	D2 | GFE
	Larvae	Diet	Diet
Listeria monocytogenes		Not detected	Not detected
Bacillus cereus		<1.0E+2	>1.5E+5
Clostridium perfringens		<1.0E+2	6.4E+3
Campylobacter			Not detected
Salmonella		Not detected	Not detected
MRSA			Not detected

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* Hygiene limit

BSF experiment – Microbiological hazards (D0)

	D3 | BRM	D4 | SMM	Legal
	Diet[1]	Diet[1]	Limit
Listeria monocytogenes	Not detected	Not detected	any
Bacillus cereus	<1.0E+2	3.8E+3	1.0E+5
Clostridium perfringens	<1.0E+2	7.7E+2	1.0E+5
Campylobacter	NA	NA	NA
Salmonella		NA	
MRSA		NA	

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* Hygiene limit

BSF experiment – Microbiological hazards (D0)

	Start
	Larvae
Listeria monocytogenes	Not detected
Bacillus cereus	<1.0E+2
Clostridium perfringens	<1.0E+2
Campylobacter	
Salmonella	Detected
MRSA	

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* Hygiene limit

BSF experiment – Microbiological hazards

	Start	D1 control			D2 GFE		
	Larvae	Diet	Larvae	Residue	Diet	Larvae	Residue
Listeria monocytogenes	no	no	no	no	no	no	No
Bacillus cereus	<1.0E+2	<1.0E+2	<1.0E+2	<1.0E+2	>1.5E+5	2.6E+4	<1.0E+2
Clostridium perfringens	<1.0E+2	<1.0E+2	<1.0E+2	<1.0E+2	6.4E+3	<1.0E+2	6.6E+2
Campylobacter					ND		
Salmonella	Detected	ND			ND		
MRSA					ND		

	D3 broiler manure			D4 supermarketmix			Legal
	Diet	Larvae	Residue	Diet	Larvae	Residue	Limit
Listeria monocytogenes	no	no	no	no	no	no	any
Bacillus cereus	<1.0E+2	<1.0E+2	<1.0E+2	3.8E+3	4.5E+3	<1.0E+2	1.0E+5
Clostridium perfringens	<1.0E+2	<1.0E+2	<1.0E+2	7.7E+2	2.1E+3	1.5E+4	1.0E+5
Aerobic bacteria	>3.0E+8	6.6E+7	>3.0E+8	1.3E+5	1.7E+8	>3.0E+8	5.0E+6*
Campylobacter	ND			ND			NA
Salmonella				ND			
MRSA				ND			

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* Hygiene limit

BSF experiment – Microbiological hazards

	Start	D1 control			D2 GFE		
	Larvae	Diet	Larvae	Residue	Diet	Larvae	Residue
Listeria monocytogenes	no	no	no	no	no	no	No
Bacillus cereus	<1.0E+2	<1.0E+2	<1.0E+2	<1.0E+2	>1.5E+5	2.6E+4	<1.0E+2
Clostridium perfringens	<1.0E+2	<1.0E+2	<1.0E+2	<1.0E+2	6.4E+3	<1.0E+2	6.6E+2
Campylobacter			NA	NA	NA	NA	NA
Salmonella	**A**	NA	**SEEP**	**SEEP**	NA	SEEP	SEEP
MRSA			NA	NA	NA	NA	NA

	D3 broiler manure			D4 supermarketmix			Legal
	Diet	Larvae	Residue	Diet	Larvae	Residue	Limit
Listeria monocytogenes	no	no	no	no	no	no	any
Bacillus cereus	<1.0E+2	<1.0E+2	<1.0E+2	3.8E+3	4.5E+3	<1.0E+2	1.0E+5
Clostridium perfringens	<1.0E+2	<1.0E+2	<1.0E+2	7.7E+2	2.1E+3	1.5E+4	1.0E+5
Aerobic bacteria	>3.0E+8	6.6E+7	>3.0E+8	1.3E+5	1.7E+8	>3.0E+8	5.0E+6*
Campylobacter	NA	NA	NA	NA	NA	NA	NA
Salmonella				NA	**SEEP**	**SEEP**	
MRSA				NA	NA	NA	

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* Hygiene limit

Salmonella enterica subsp. *enterica* Serovar Putten

	Start
	Larvae
Listeria monocytogenes	Not detected
Bacillus cereus	<1.0E+2
Clostridium perfringens	<1.0E+2
Campylobacter	
Salmonella	Detected
MRSA	

- *S. Putten*
  - 1964: reptiles (lizards)

  - Potentially pathogen for humans
    - Case study 2008 (France) -> 9 people after eating raw beef

  - RIVM:
    - since 2010 approx. 30x detected:
    - from animal sources, but also a number of human strains
- A high prevalence of *S.* Putten have been recorded from
  - swine farms in Canada and,
  - animal feed material in Sweden

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BSF experiment – Microbiological hazards

	Start	D1 control			D2 GFE		
	Larvae	Diet	Larvae	Residue	Diet	Larvae	Residue
Aerobic bacteria	>3.0E+8	1.4E+8	1.6E+8	>3.0E+8	>3.0E+8	1.1E+8	>3.0E+8
Aerobic spore count			1.4x10^6	1.58x10^7	2.2x10^6	5.8x10^7	5.2x10^8
Anaerobic spore count			4.5x10^5	1.5x10^7	5.3x10^5	6.2x10^7	1.5x10^8

	D3 broiler manure			D4 supermarketmix			Legal
	Diet	Larvae	Residue	Diet	Larvae	Residue	Limit
Aerobic bacteria	>3.0E+8	6.6E+7	>3.0E+8	1.3E+5	1.7E+8	>3.0E+8	5.0E+6*
Aerobic spore count	6.1x10^5	3.0x10^3	8.4x10^4	2.3x10^4	1.36x10^7	1.43x10^7	
Anaerobic spore count	2.3x10^2	3.2x10^3	5.5x10^4	4.1x10^3	4.4x10^6	1.5x10^6	

* Hygiene limit

Overall conclusion

- Most contaminants much lower concentrations in the insects compared to the feed
- Cadmium and some other metals accumulate, but remain at a low level