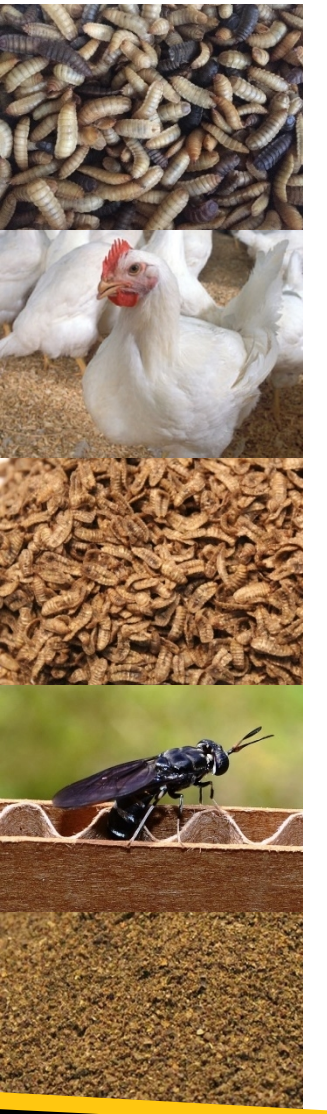


Anticoccidial and Antibacterial Activities of Black Soldier Fly Extract

Laura Sedano[1], Emilie Chambellon[1], Françoise I. Bussière[1], Maryline Vian[2], Côme Guidou[3], Christophe Trespuech[3], Emmanuelle Helloin[1], Anne Silvestre[1]

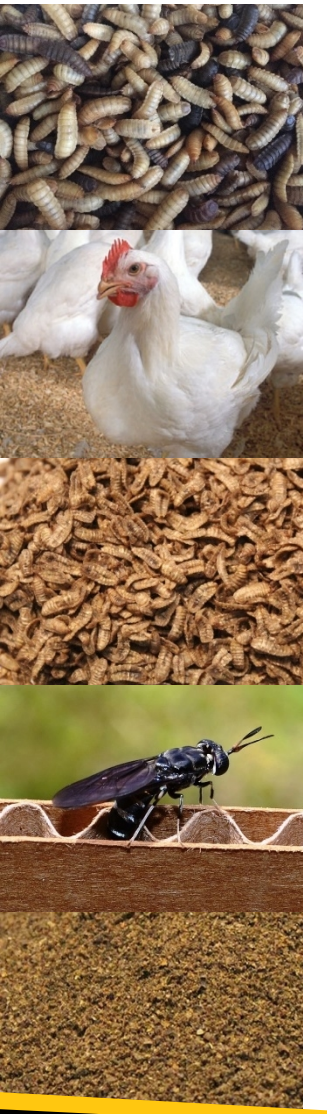
1 INRAE, Université de Tours, UMR ISP, Nouzilly, France

2 INRAE, Université Avignon, UMR SQPOV, Avignon, France

3 MUTATEC, Châteaurenard, France

Anne.Silvestre@inrae.fr

Context & Objectives



Poultry, 1st source of protein in human alimentation

Soybean meal: imported from South America (GMO, environmental impact…) but competitive protein source for birds

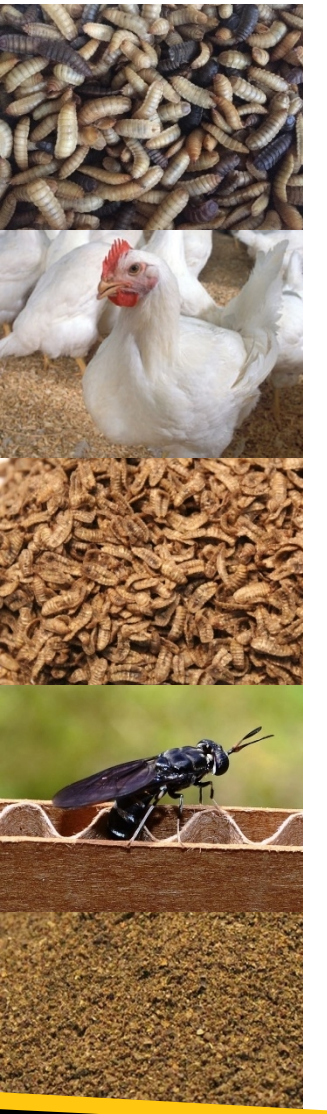
Good alternative, as natural component of birds' diet, convert organic wastes and by-products, circular economy, entomo-conversion, possible effect for poultry health and welfare

CircularEconomy

Organic farming, exposure to wild animals and their pathogens, eradicate AB

Determine the antimicrobial properties of insect larvae fed to broilers

Black Soldier Fly Larvae (BSFL) extracts



Living larvae from Mutatec

Blanched, freeze-dried larvae
MeOH extraction

Extract 1 (lipids / MeOH)

Defatted larvae – Alkaline Extraction

Heat-dried, pressed larvae
Heat Extraction

Extract 4 (lipids / MeOH)

Extract 3 (proteins / H_2O)

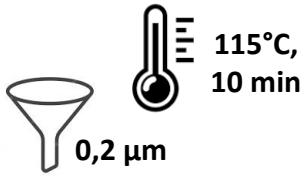
7-10% mb lipids

Extract 2 (proteins / H_2O)

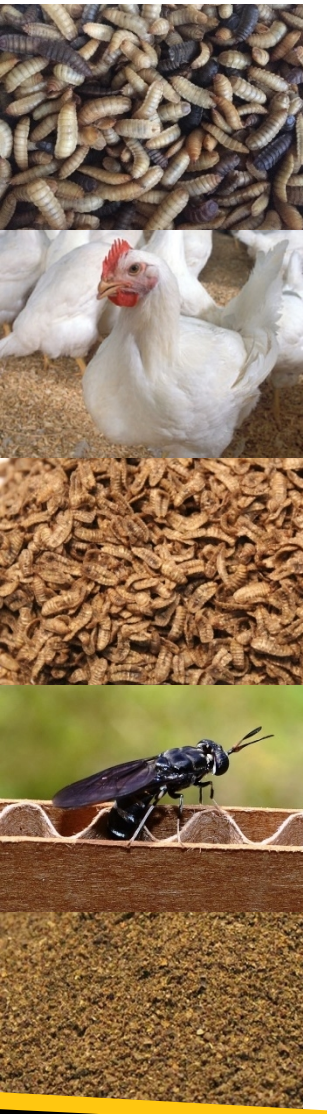
Deffated insect flour

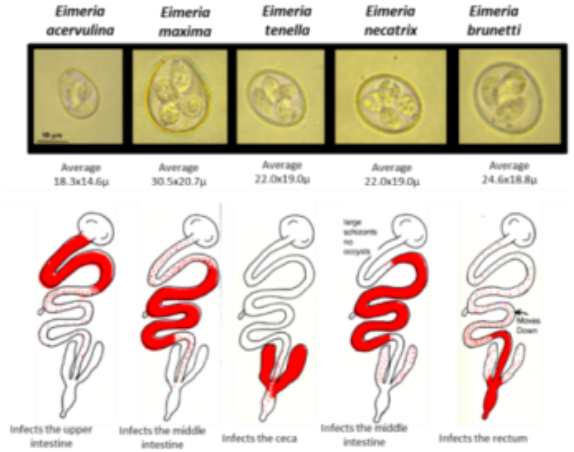
Batch 1 - Sterilized extracts	20 g/L eq. dry matter	**1:2 dilutions**
Batch 2 - Sterilized extracts	35 g/L eq. dry matter	**1:2 dilutions**

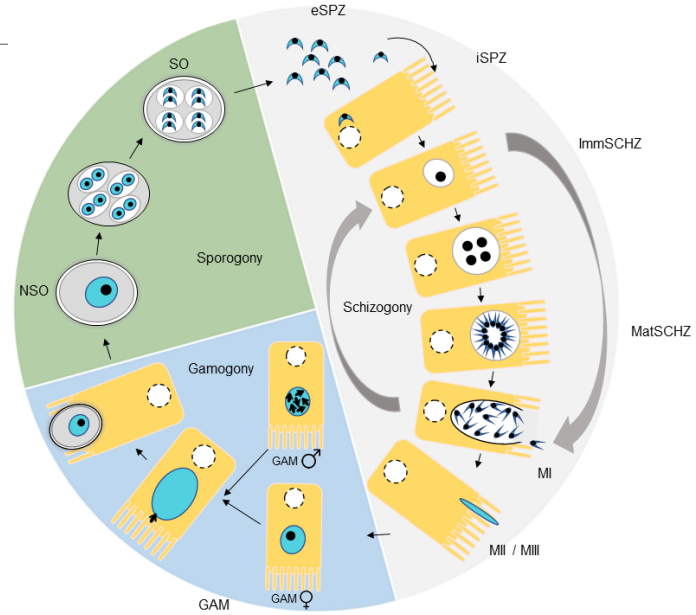
Batch 1 - Native	20 g/L eq. dry matter	**1:10 dilutions**
Batch 2 - Sterilized extracts	35 g/L eq. dry matter	**1:10 dilutions**

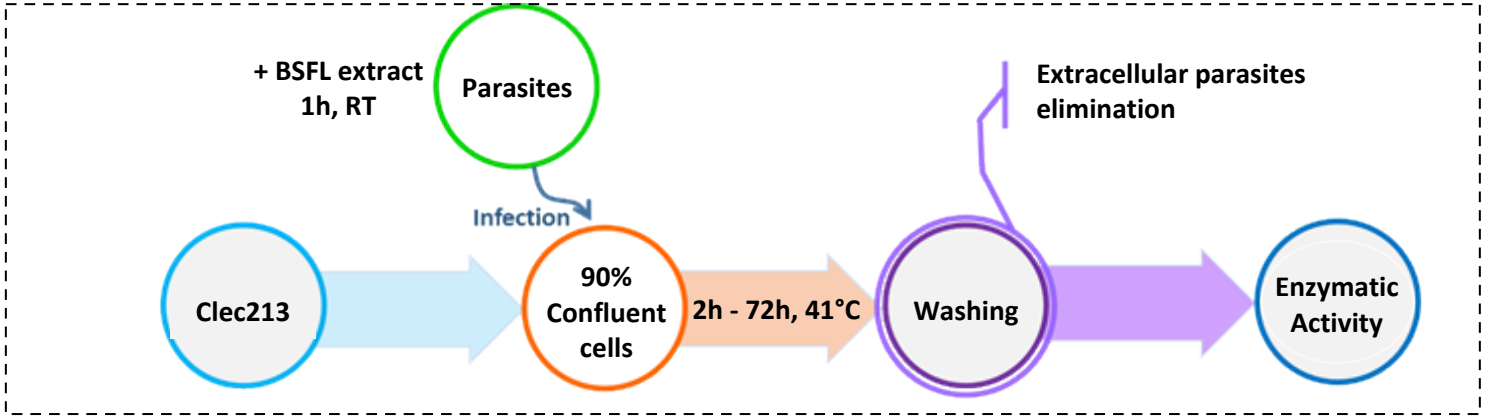


1. Experimental evaluation of anticoccidial properties



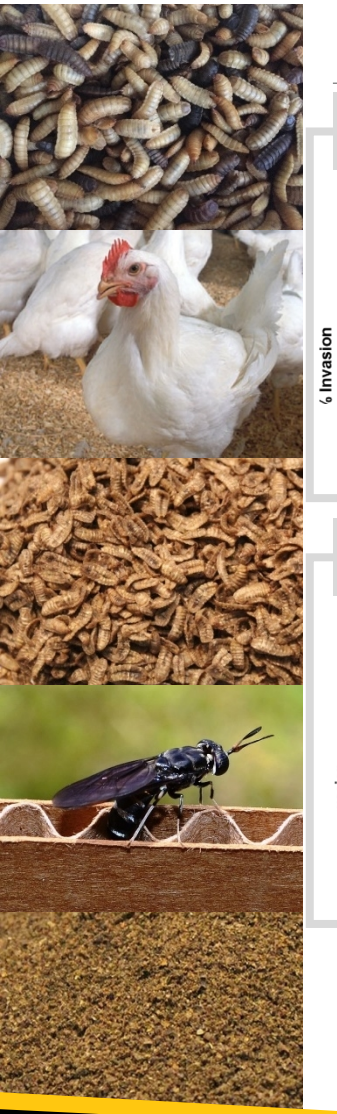


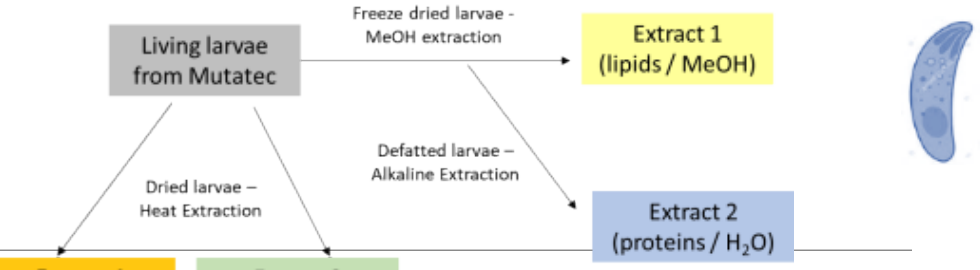




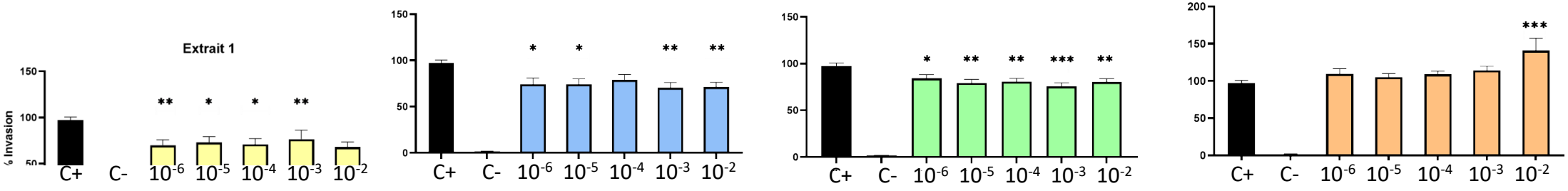
- Control + : solvent
- Control – : heat killed parasites

1. Anticoccidial properties (invasion)

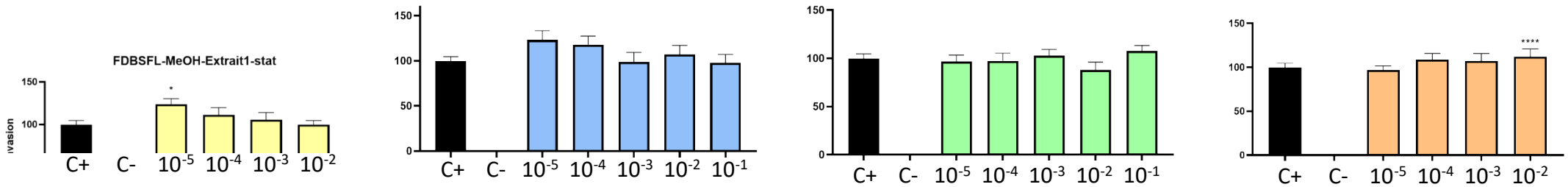




Batch 1

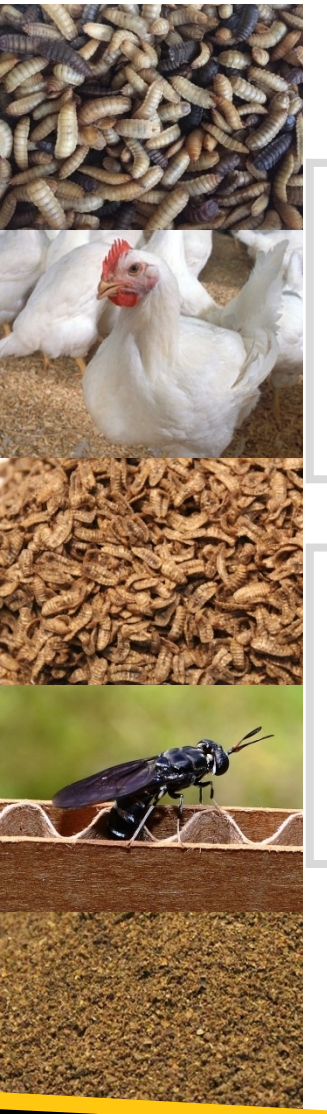


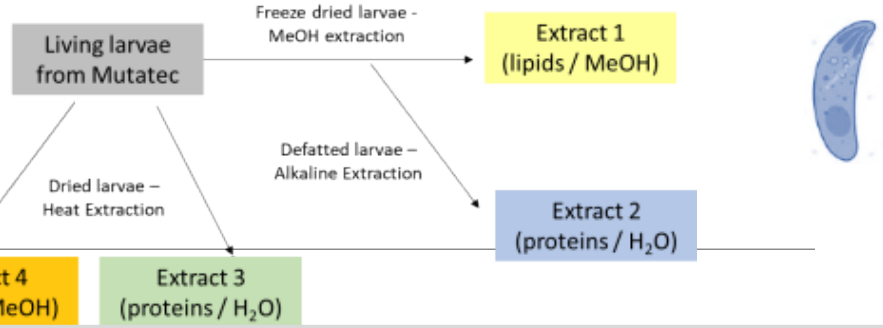
Batch 2



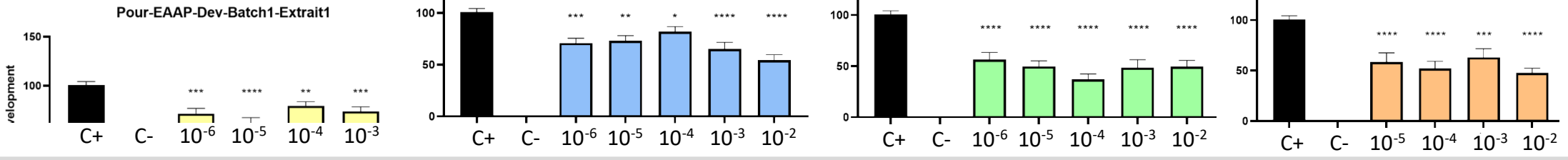
Batch 1 - Native	20 g/L eq. dry matter	**1:10 dilutions**
Batch 2 - Sterilized extracts	35 g/L eq. dry matter	**1:10 dilutions**

1. Anticoccidial properties (development)

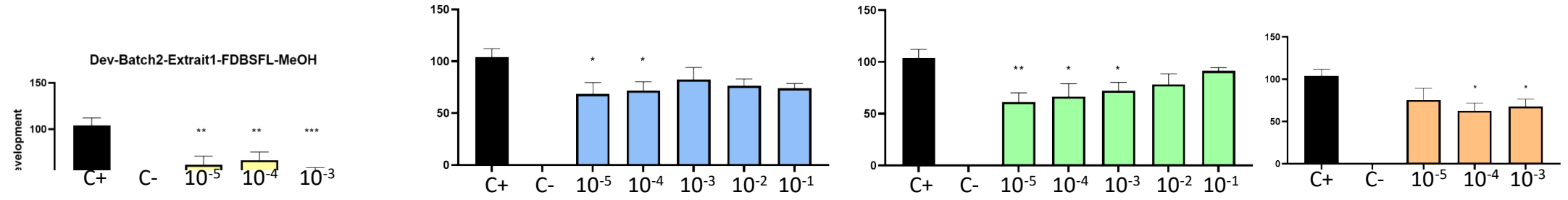




Batch 1

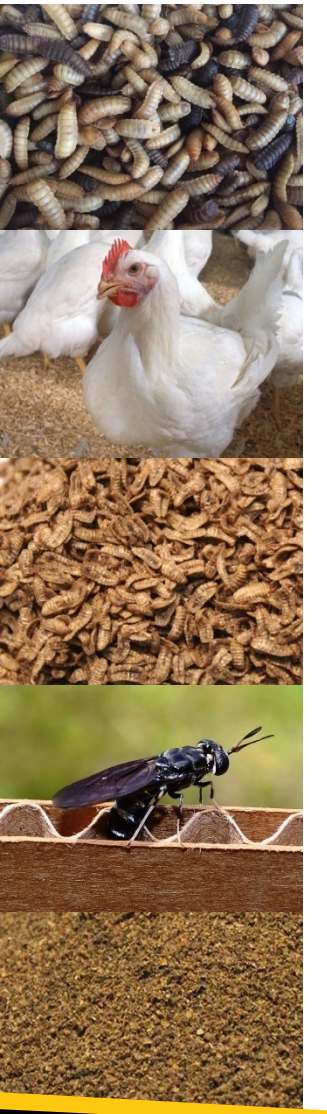


Batch 2



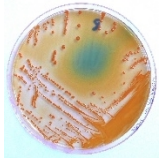
Batch 1 - Native	20 g/L eq. dry matter	**1:10 dilutions**
Batch 2 - Sterilized extracts	35 g/L eq. dry matter	**1:10 dilutions**

2. Experimental process for antibacterial properties evaluation

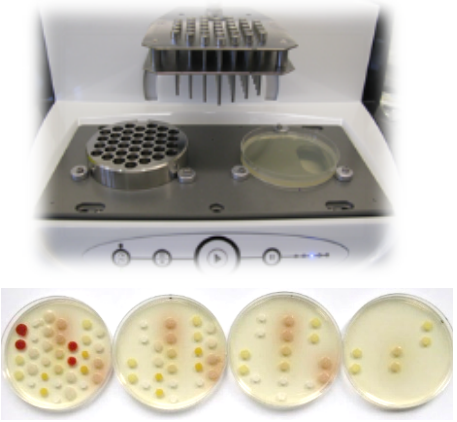


Minimal Inhibitory Concentrations (MIC) of each extract/bacterial strain couple are determined in agar plates, according to a **standardized procedure**:

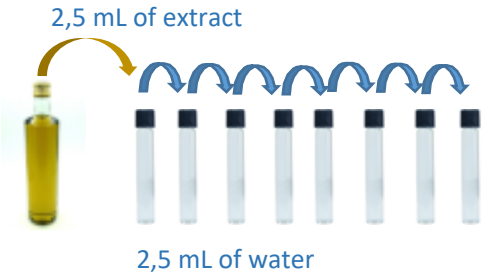
Calibrated bacterial inocula



0,5 Mc Farland
1/10



Dilution range of the antimicrobial agent



2 mL of each concentration of extract
+ 18 mL of Mueller Hinton agar

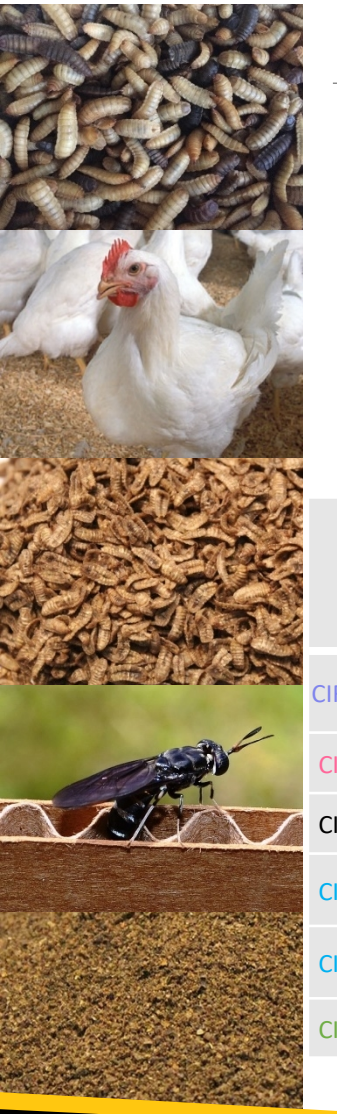
MIC is the lower concentration of antimicrobial agent that completely inhibits bacterial growth

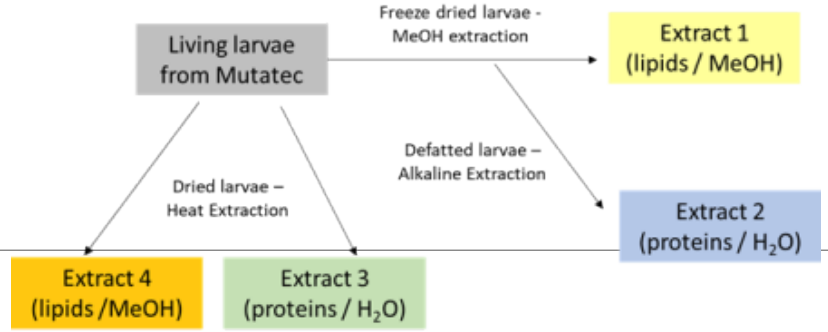
Controls are used to assess the absence of antimicrobial activity of extraction solvents

Target of a panel of 36 bacterial strains that threaten livestock production :

- **poultry** (turkey, rabbit, chicken, duck), **cattle, pigs and Fish**
- 18 **non demanding growth strains** (37°C, 24h)
- 18 **demanding growth strains** (+serum, 37°C + 5% CO_2, 48h)
- with **varied antibiotic resistance profiles**

2. Antibacterial properties evaluation





- No antibacterial activity of **protein-concentrated extracts**
- Antibacterial activity of **lipid-concentrated extracts 0,7 ≤ MIC ≤ 5,7 g dry matter/L,** on certain growth-demanding strains (*Pasteurella multocida, Corynebacterium bovis, Streptococcus suis, Riemerella anatipestifer, Trueperella pyogenes)*

CIRM-BP Ref.	Genus/species	Bacterial cell wall	Animal origin / pathology	AMR profile	MIC Extract 1 g/L dry matter	MIC Extract 4 g/L dry matter
CIRMBP-1183	*Riemerella anatipestifer*	Gram -	Duck / Riemerellosis	S	0,70	2,78
CIRMBP-971	*Trueperella pyogenes*	Gram +	Pig / infectious arthritis	R	2,83	2,78
CIRMBP-985	***Streptocoque suis II***	**Gram +**	**Pig /piglet meningitis**	**R**	**2,83**	**/**
CIRMBP-884	*Pasteurella multocida subsp multocida*	Gram -	Rabbit / Pasteurellosis	ND	2,83	5,56
CIRMBP-873	*Pasteurella multocida subsp septica*	Gram -	Rabbit / Pasteurellosis	S	2,83	5,56
CIRMBP-975	*Corynebacterium bovis*	Gram +	Bovine / Mastitis	S	5,66	/

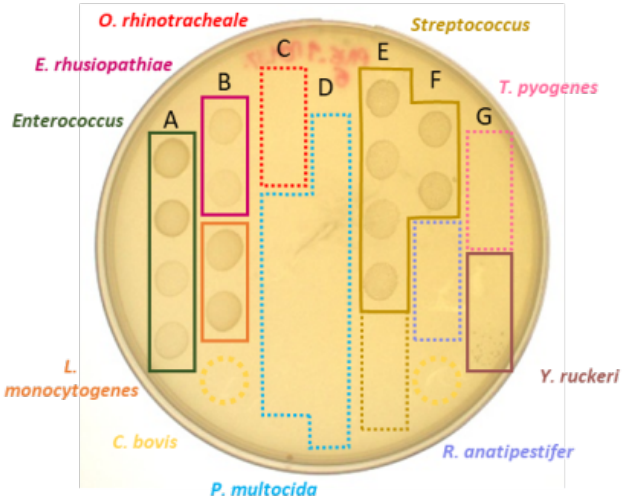
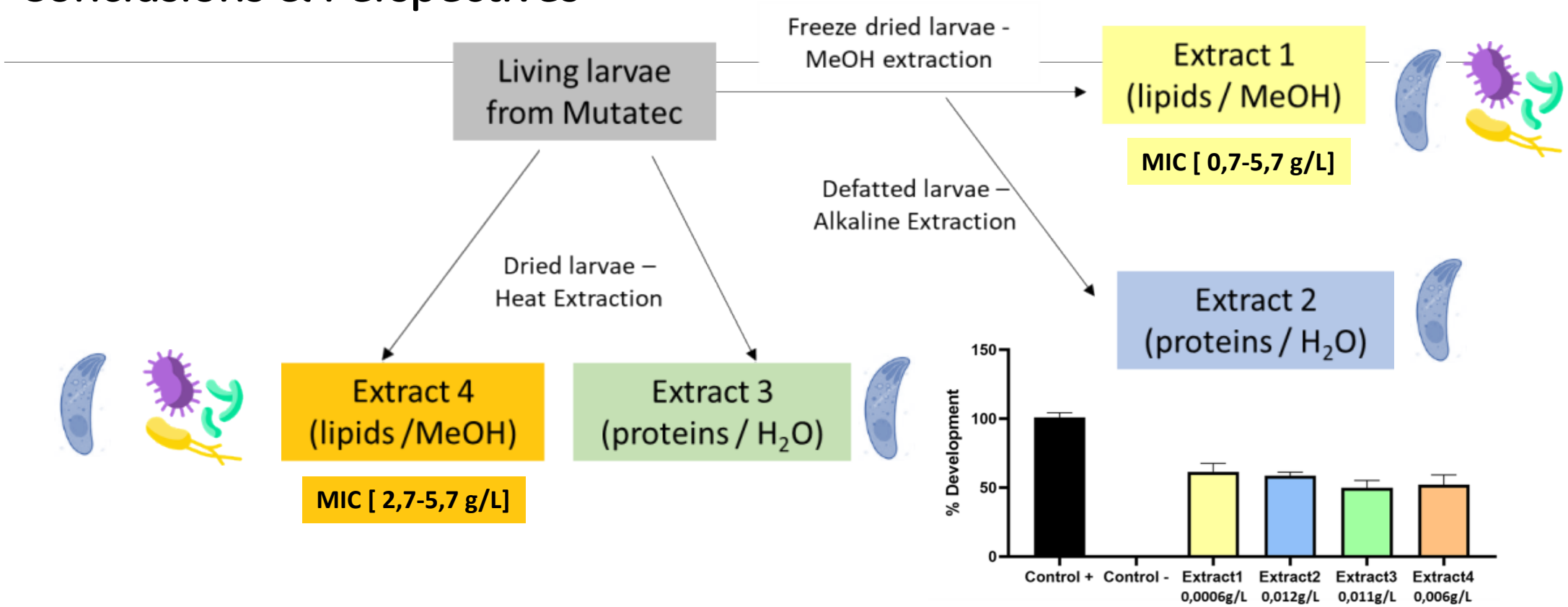


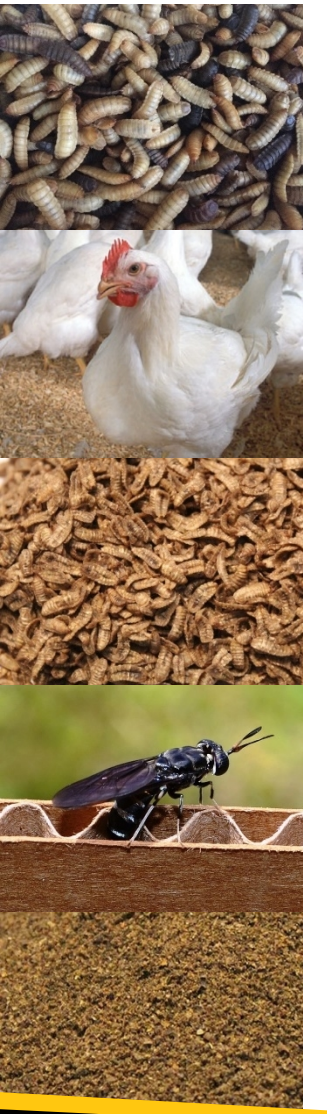
Illustration : Extract 1, 5,66 g/L

Conclusions & Perspectives



- **Lipids extracts were more efficient in our conditions (antiparasitic + antibacterial activity), at lower doses**
- **Inhibition of intracellular development**: possible effect on the parasite or on the host cell (proteomic analysis)
- Immunomodulatory activities remain to be evaluated
- The antimicrobial effects of BSFL could improve the health and the immune response of birds, when facing sanitary challenges.

Acknowledgments



ITAVI

Technical institute for poultry

AVIGNON UNIVERSITÉ

UMR SQPOV
BSFL extracts

INRAE

UMR BOA (Avian Biology & Poultry Research)
UMR ISP (Infectious Diseases & Public Health)
UE PEAT (Tours poultry experimentation center)
UMR TOXALIM (Research Centre in Food Toxicology)

INP TOULOUSE PURPAN ÉCOLE D'INGÉNIEURS

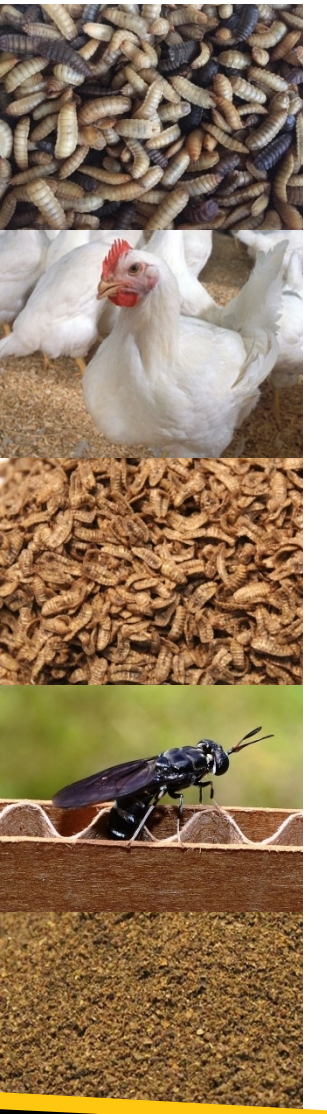
Sciences du vivant | Agriculture
Agroalimentaire | Marketing | Management

MUTATEC

Private company - BSF products

Partner Green

Batch 1, 20 g dry matter / L



Process MUTATEC

Live BSFL → Drying → Pressing → **BSFL crude Oil*** / **BSFL protein meal****

- **Mutatec protein concentrate:** 4 g of **BSFL protein meal**** in 200 mL dH_2O (filtered)
  - **Extract 1:** 4 g of **BSFL crude oil*** in 200 mL DMSO
  - **Extract 2:** 4 g of **solvent defatted BSFL flour***** in 200 mL dH_2O (filtered)

Process GREEN

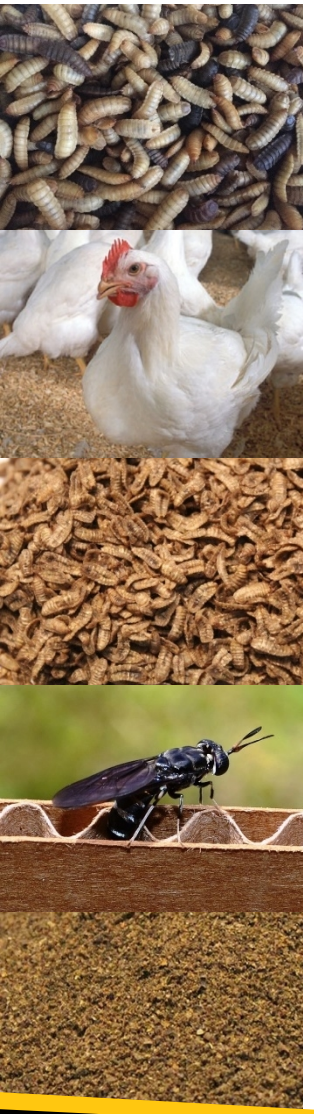
Live BSFL → Blanching → Freezing and freeze-drying → Oil extraction (hexane) → **Solvent exracted oil** / **Defatted BSFL flour*****

Extract 4

Extract 3

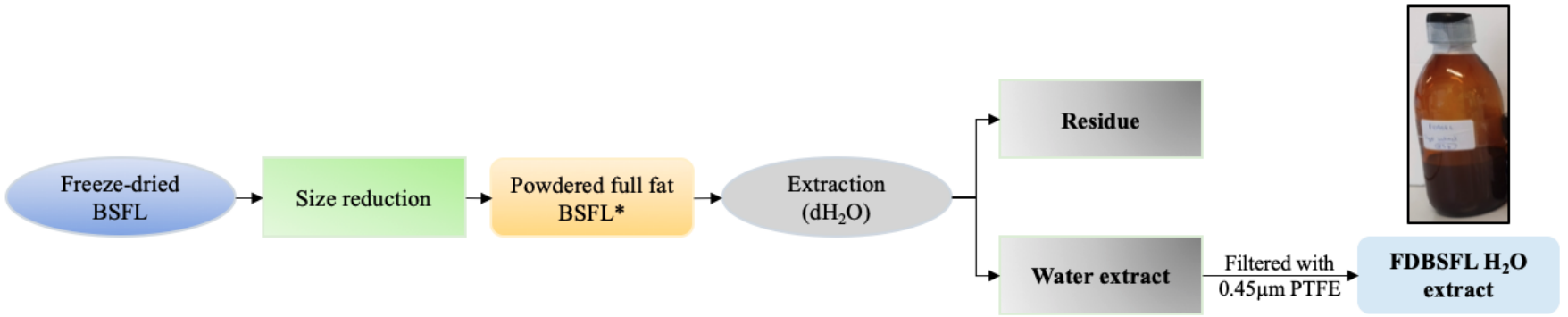
Extract 1

Extract 2



Batch 2, 37 g dry matter / L

Process GREEN: FDBSFL water extract



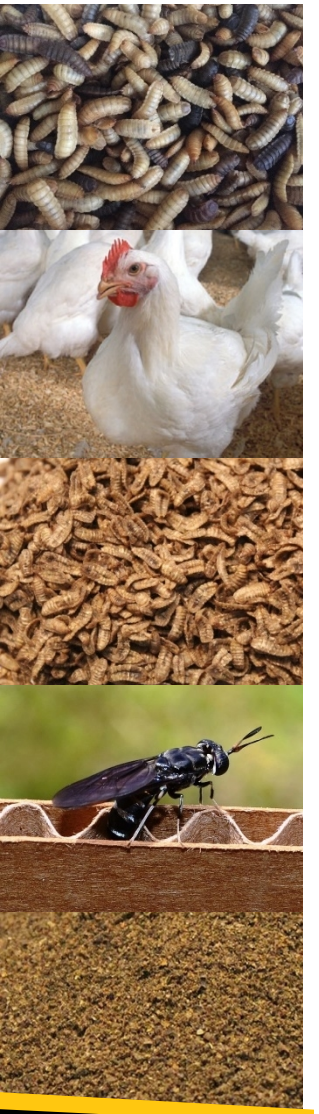
Extract 2

- 50 g of powdered full fat BSFL* was mixed with 250 mL of distilled water at room temperature for 1h (magnetic-stirring).
- After extraction, the mixture was filtered multiple times (x 3) to get clear water extract.
- The water extracts were filtered through 0.45µm PTFE filters before bottling.
- The extract was labelled "FDBSFL H_2O extract" and stored at -18°C.

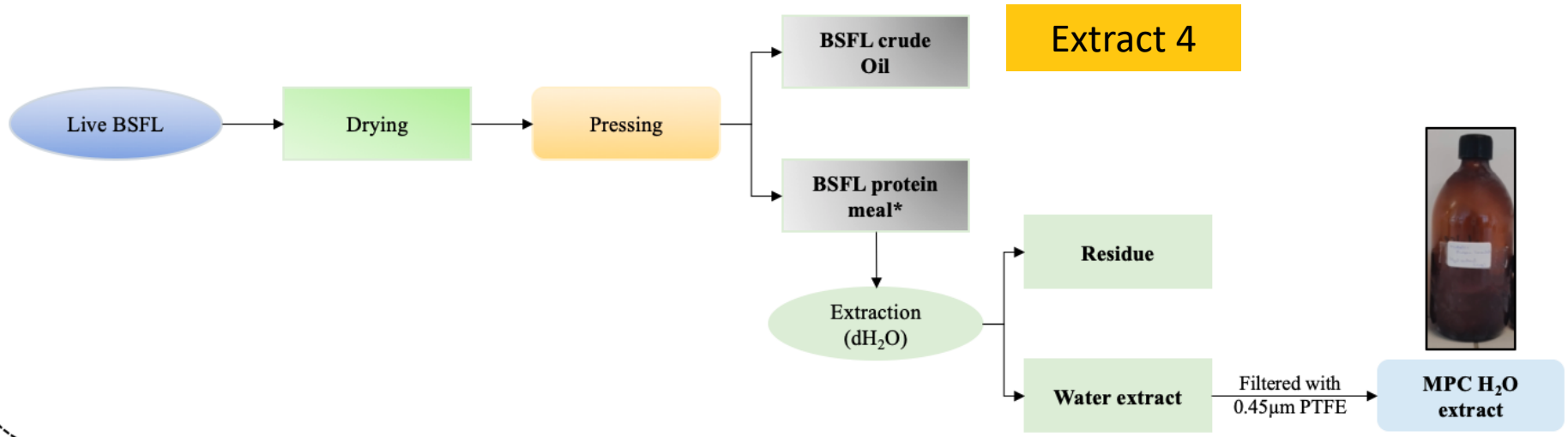
Dry matter = **3.7 %**

Batch 2, 34 g dry matter / L

Partner Green



Process MUTATEC – GREEN: MPC water extract



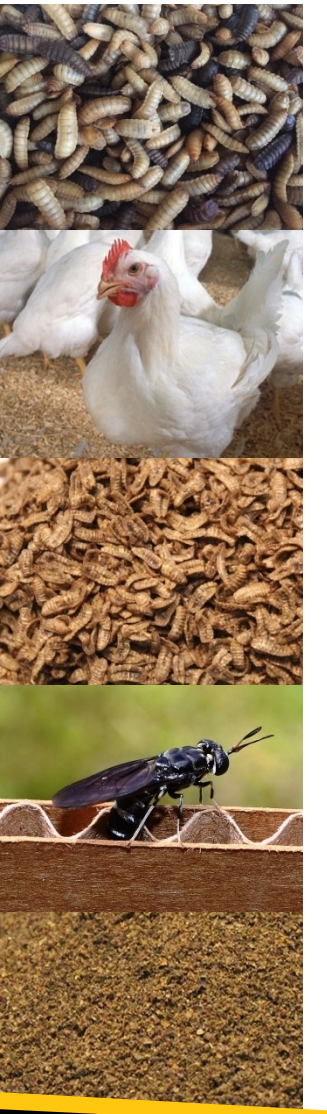
Extract 4

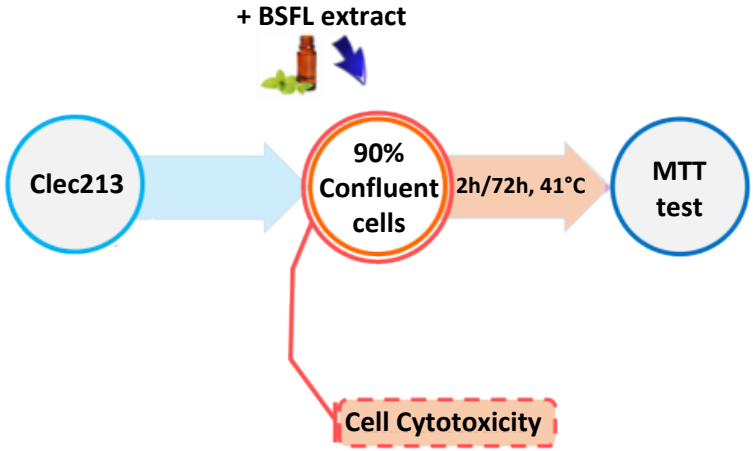
Extract 3

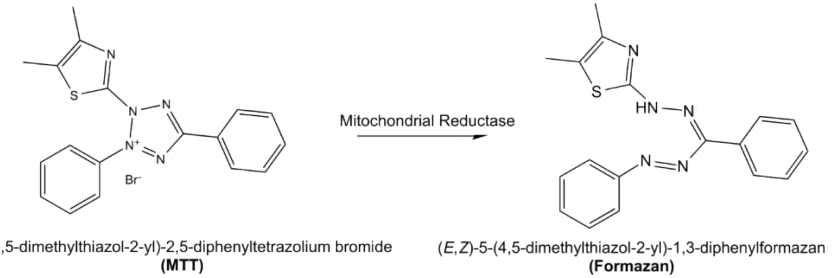
- 50 g of powdered BSFL protein meal* was mixed with 250 mL of distilled water at room temperature for 1h (magnetic-stirring).
- After extraction, the mixture was filtered multiple times (x 3) to get clear water extract.
- The water extracts were filtered through 0.45µm PTFE filters before bottling.
- The extract was labelled "Mutatec protein concentrate H_2O extract" and stored at -18°C.

Dry matter = 3.4 %

Cytotoxicity evaluation

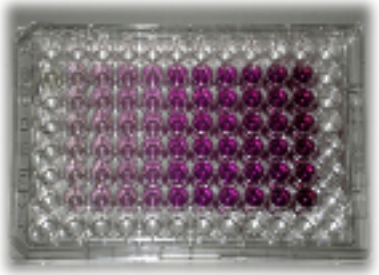






3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
(MTT)

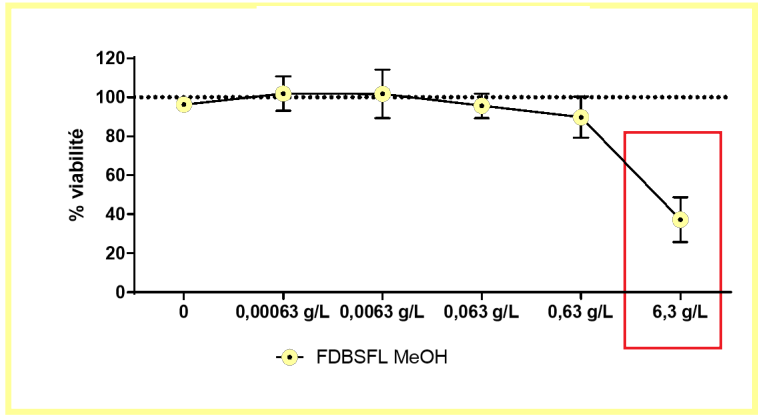
(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan
(Formazan)



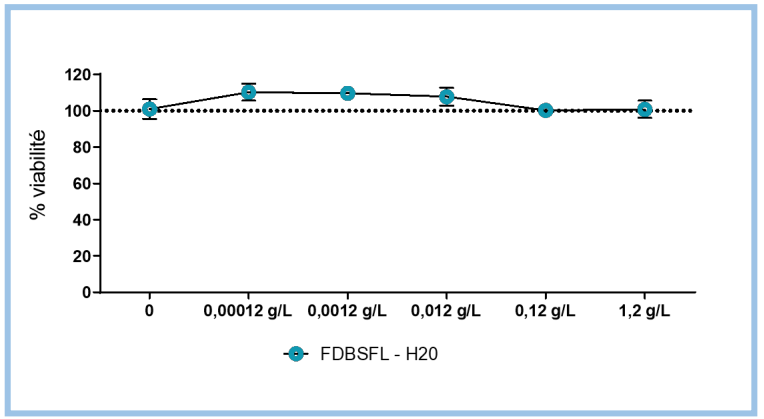
CLEC213 : avian epithelial cells

Cytotoxicity evaluation

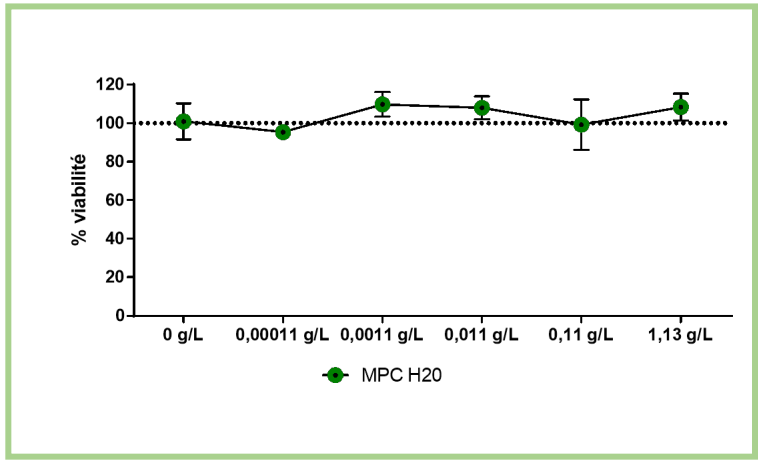
Extract 1



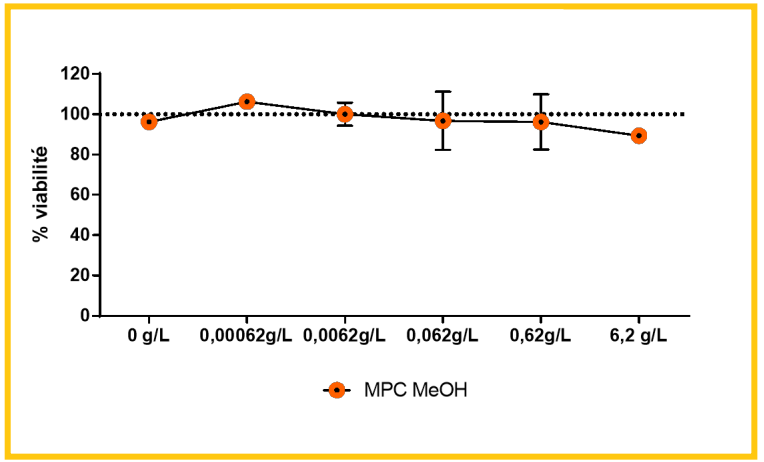
Extract 2

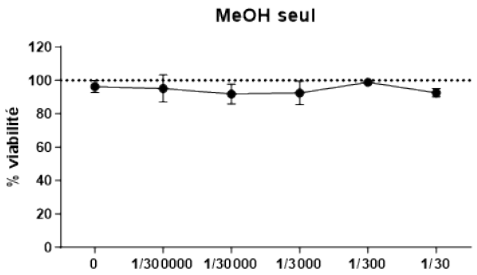


Extract 3



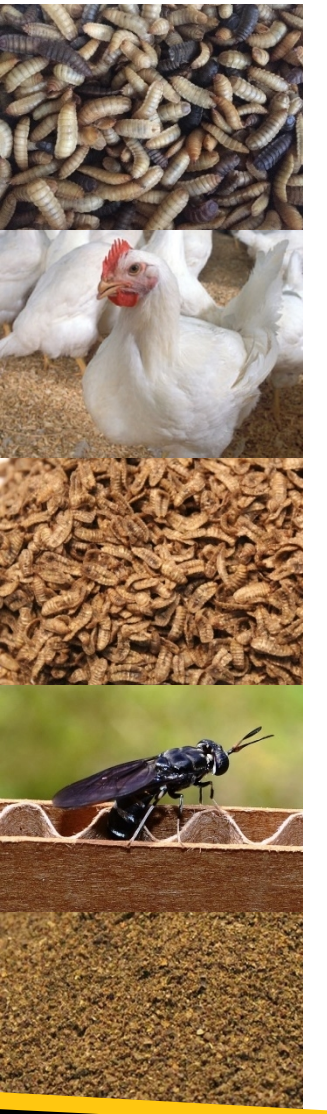
Extract 4



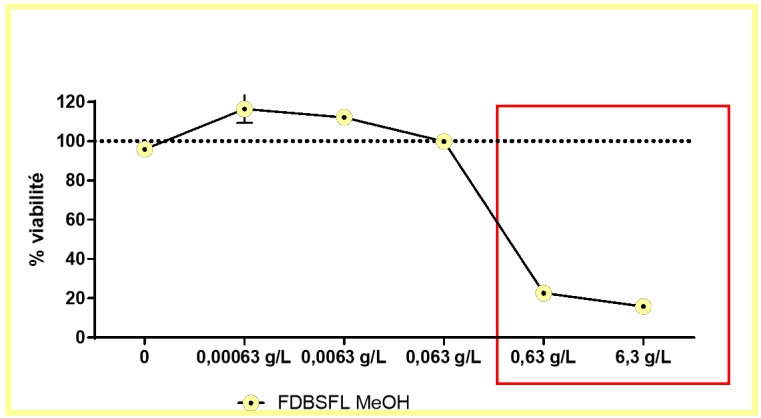


2h post infection = Invasion

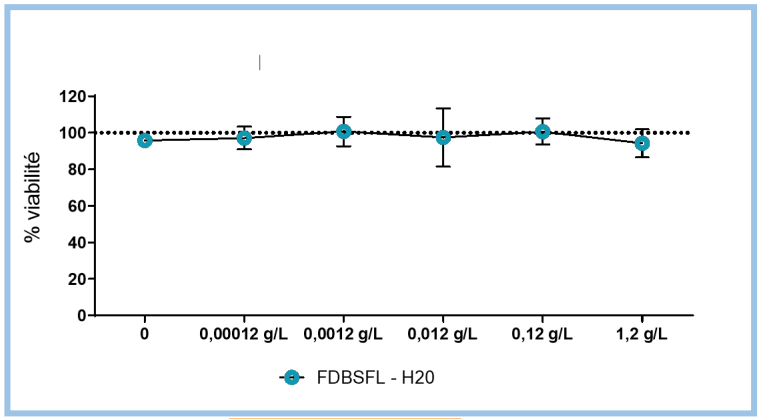
Cytotoxicity evaluation



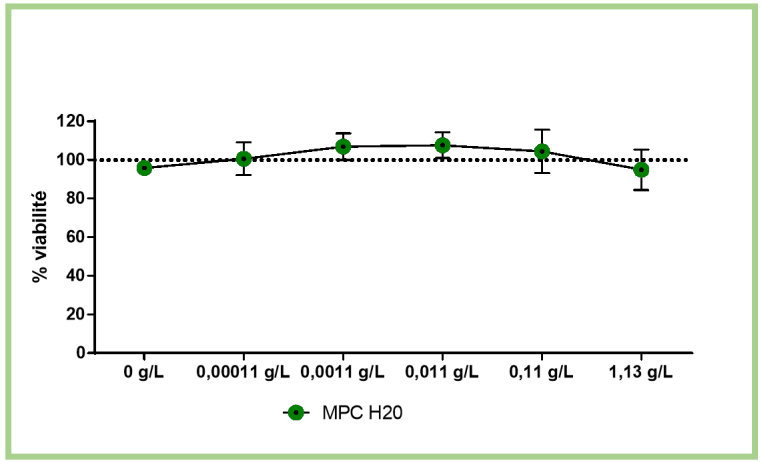
Extract 1



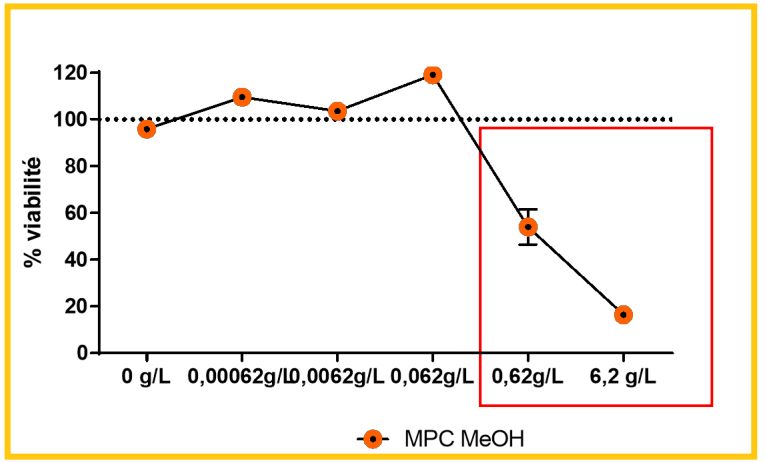
Extract 2



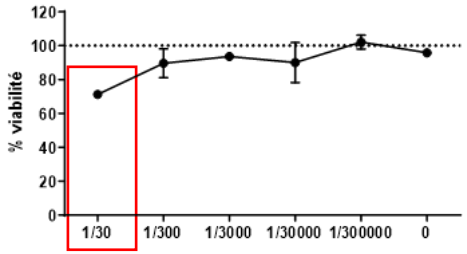
Extract 3



Extract 4

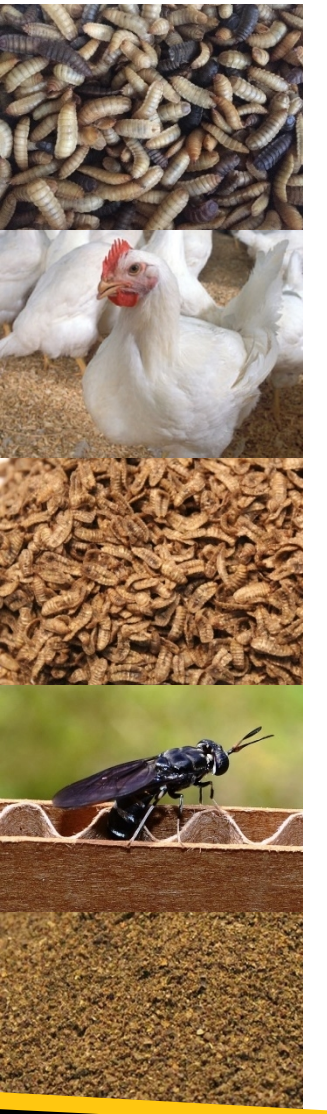


MeOH seul

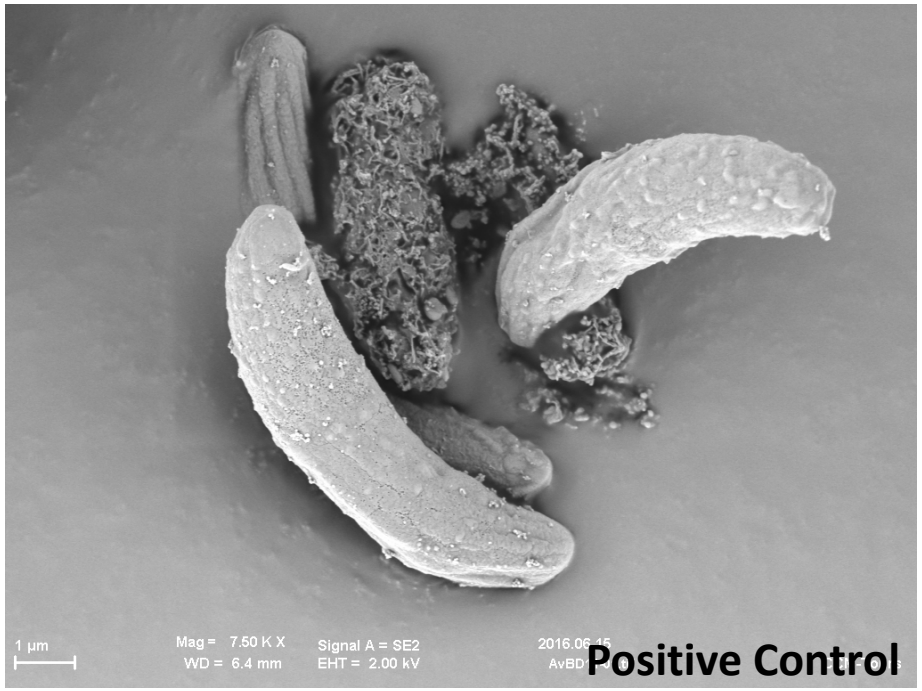


72h post infection = Development

Scanning Electron Microscopy (spz incubated with extract)

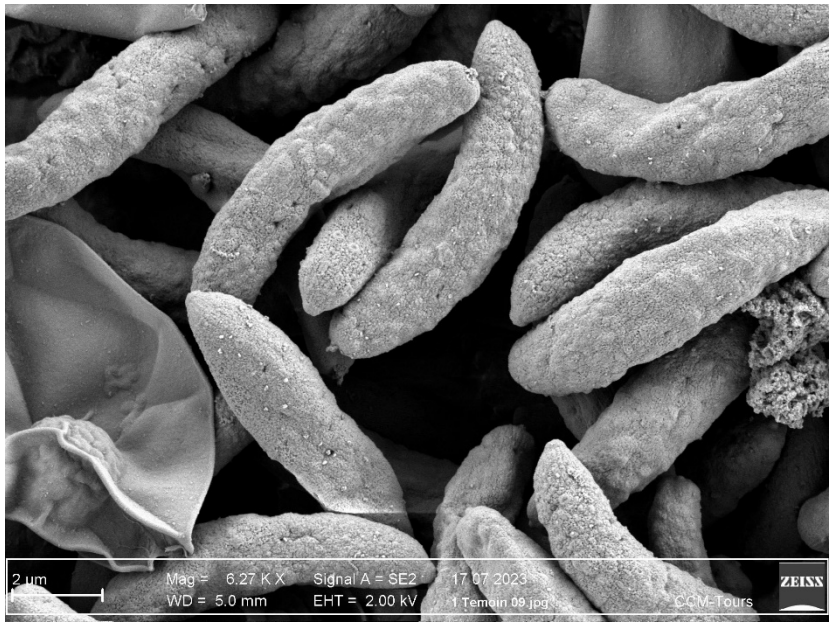


Negative Control (PBS)

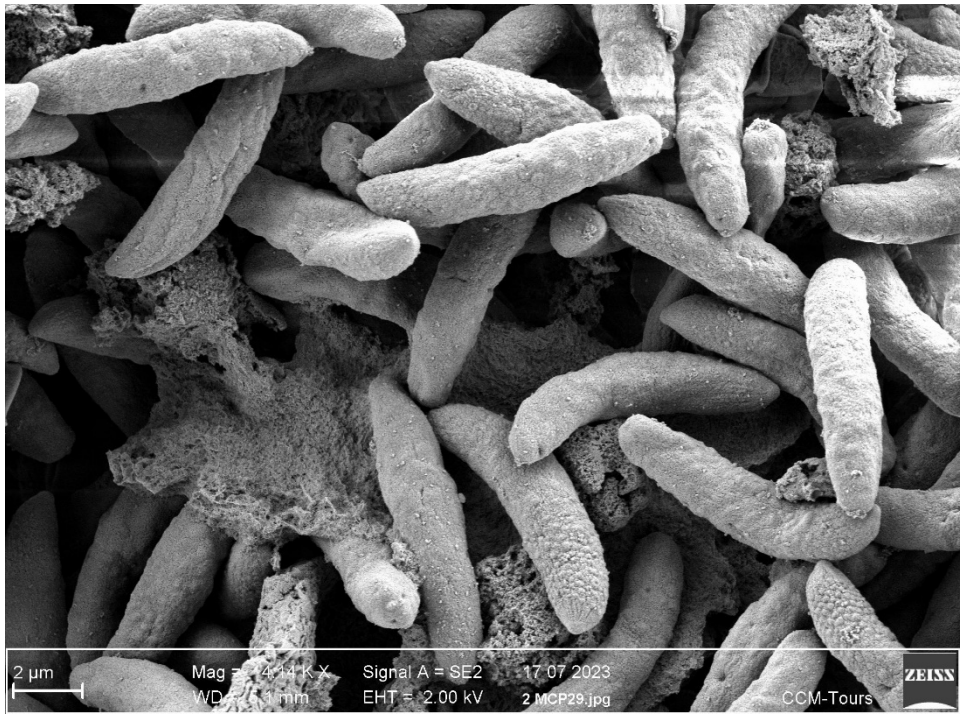


Positive Control (PAM AvBD11)

Scanning Electron Microscopy (spz incubated with extract)

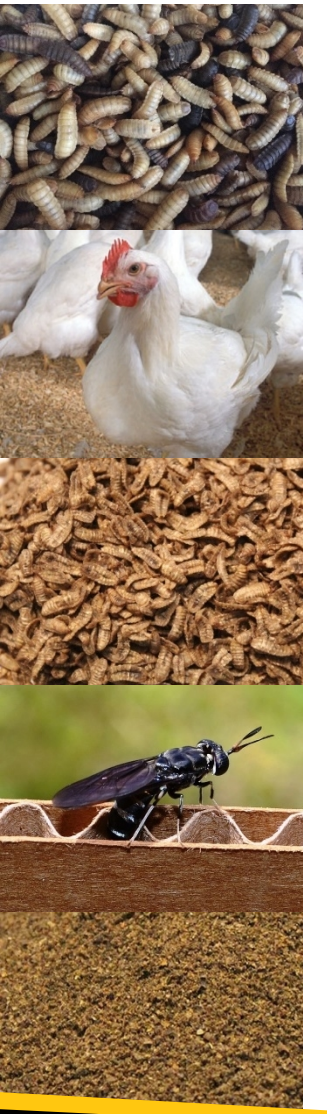


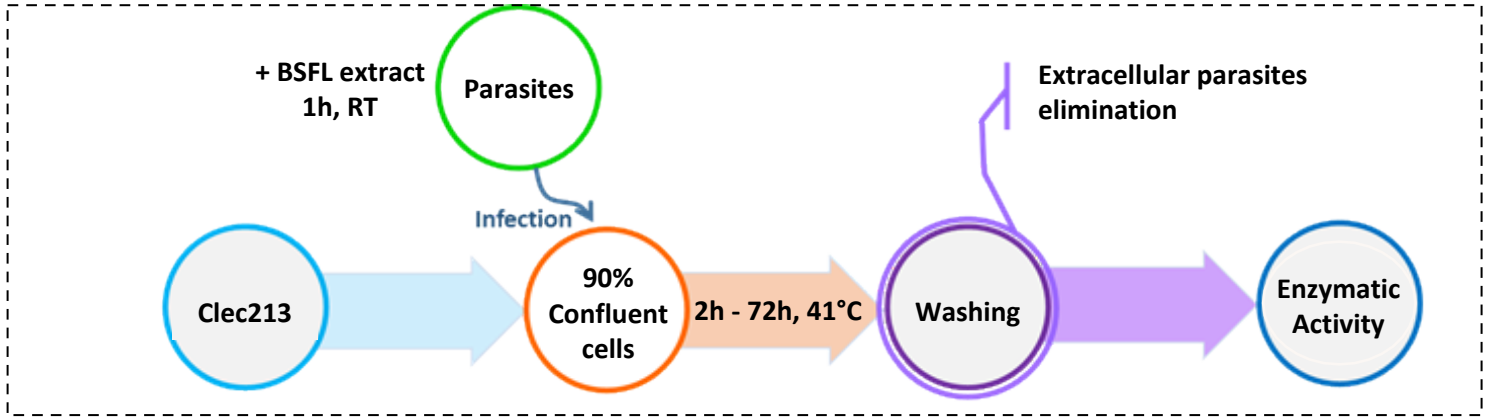
Control (PBS)



Extract N°4

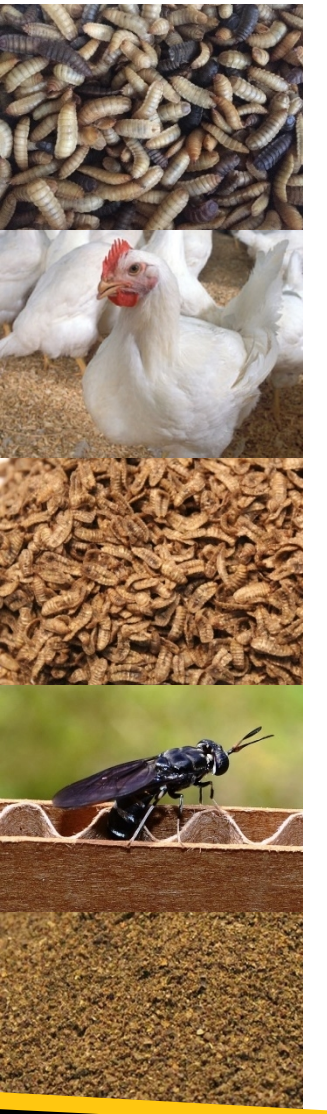
1. Experimental process for anticoccidial evaluation





- Control + : solvent
- Control – : heat killed parasites

Insects for animal feed, to improve protein autonomy and animal welfare.



Authorized: living insects, lipid extract, and recently protein extract (EU 2021/1372)

Defatted protein extract (85% proteins 15% lipids): higher protein ratio than soybean, and large proportion of saturated fatty acids (50% lauric acid)

Chitin, lauric acid and AMP composition/effect: poorly described