



Effect of agro-industrial by-products-based diet on the gene expression of antimicrobial peptides in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae

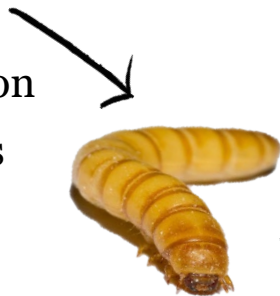
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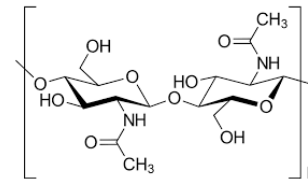


Diet formulation

Microbiological contamination
Minerals and contaminants

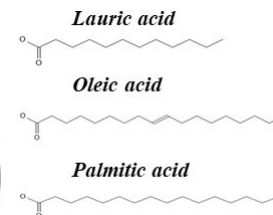


Chitin



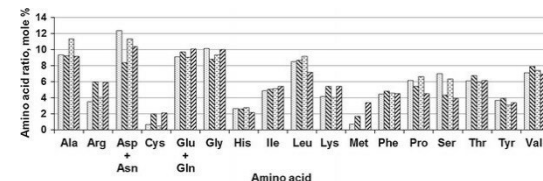
Lipid content

Fatty acid profile



Protein content

Amminoacidic profile



Chemical and microbiological hazards

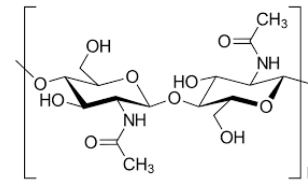


Diet formulation

Microbiological contamination
Minerals and contaminants

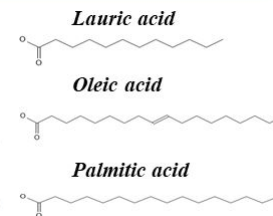


Chitin



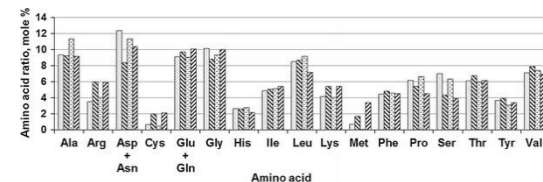
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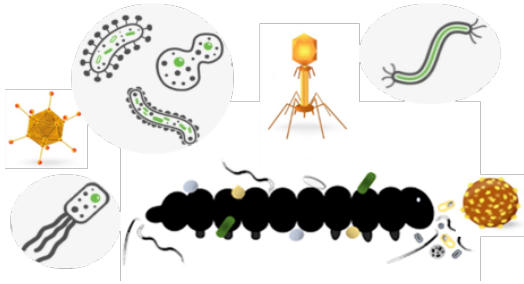


Chemical and microbiological hazards



Insect immune system?

INSECT IMMUNE SYSTEM



Regulates interactions with other organisms

- Harmful organisms
- Organisms that can be tolerated
- Beneficial microorganisms

**Complex mechanisms activated by different types of signals
and that can be independently regulated**

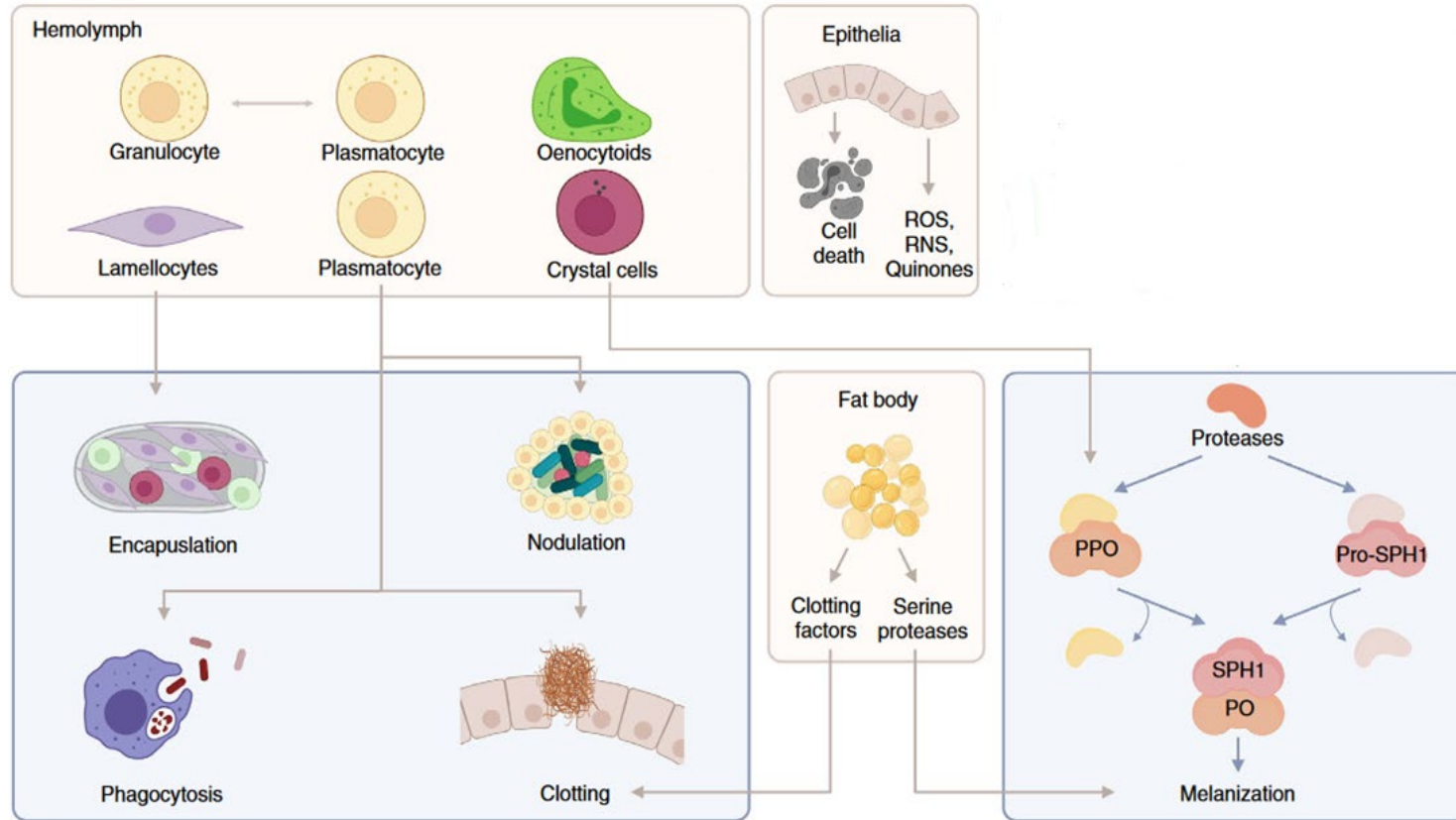


Cellular responses



Humoral responses

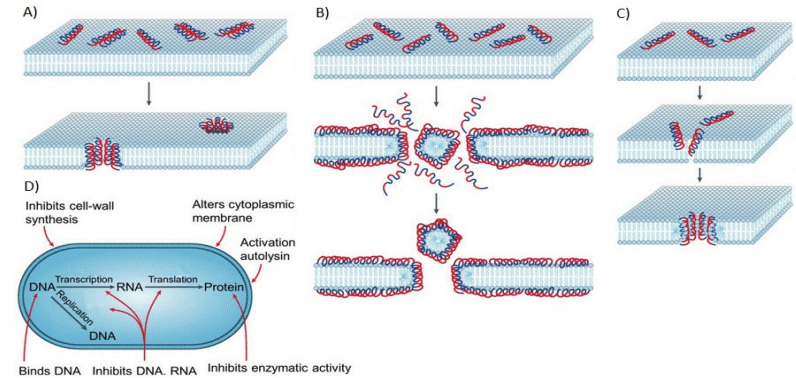
Cellular responses - hemocytes



Modified from Eleftherianos *et al.*, 2021. *Immunology*, 164:401-432

Humoral responses

➤ Antimicrobial peptides (AMPs)



Broden *et al.*, 2005. *Nat. Rev. Microbiol*, 3(3): 238-250

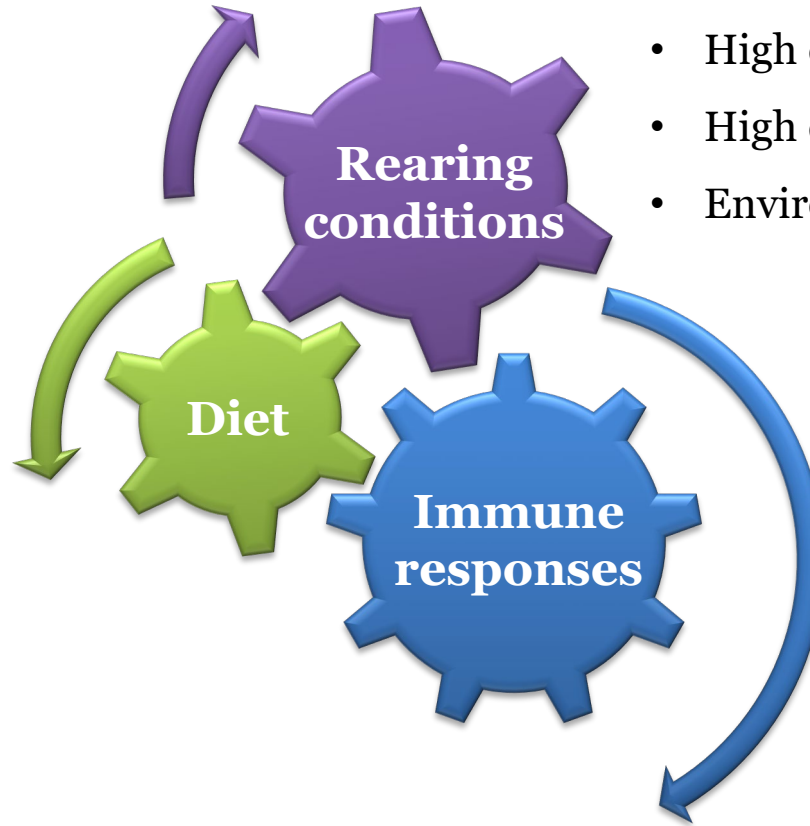
- Reactive oxygen and reactive nitrogen intermediates
- Enzyme cascades (i.e. proPO system)
- Coagulation and melanization



Aperis *et al.*, 2007. *Microbes infect.*, 9(6): 729-734

Insect immune system

- Substrate availability
- Protein/carbohydrate ratio
- Other nutrients



- High density
- High consanguinity
- Environmental conditions

T. molitor entomopathogens



- Opportunistic bacteria
→ *Pseudomonas* and *Serratia*
- Entomopathogenic fungi
→ *Metarhizium* and *Beauveria*
- Parasites → Gregarines and Nematodes
- Densovirus

Armién et al., 2023. *Vet. Pathol.*, 60(5), 689-703



Strengthen the immune system of insects in mass rearing



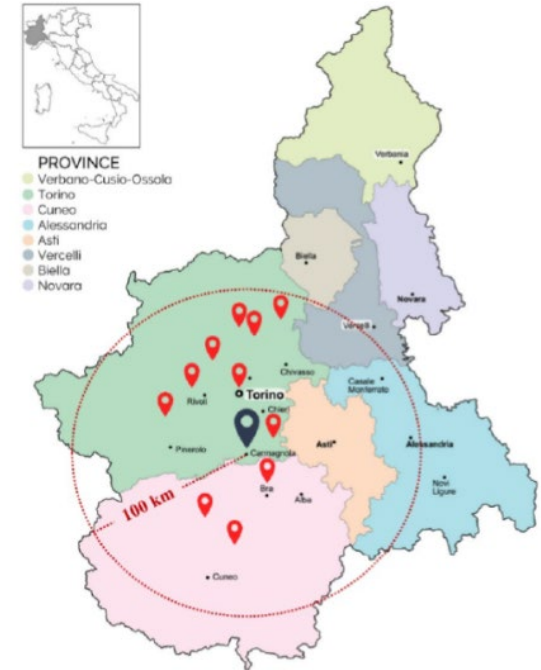
Lower risk of disease in the rearing systems

Aims of the research

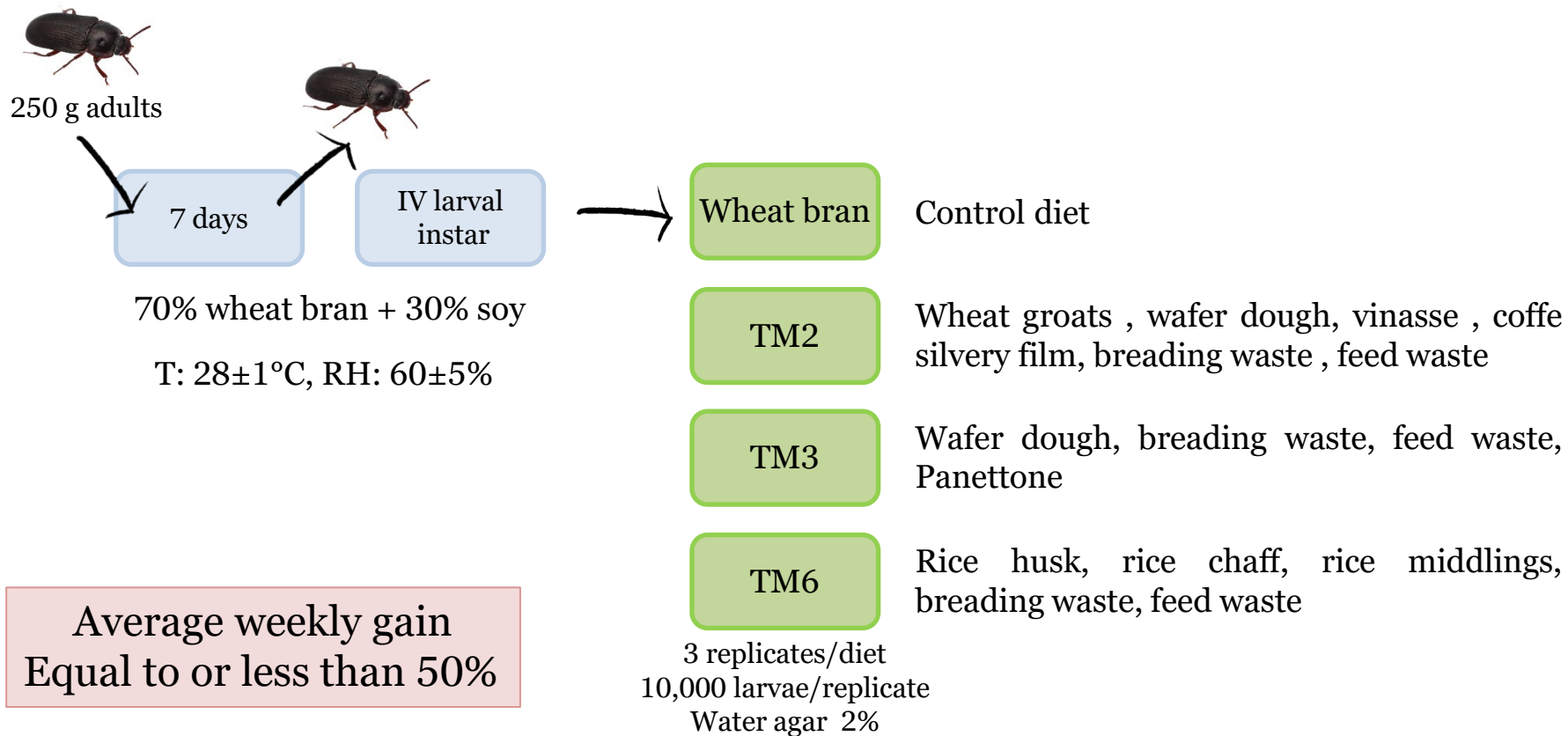


Effect of **agro-industrial by-products-based diet** on the gene expression of AMPs in *Tenebrio molitor* larvae

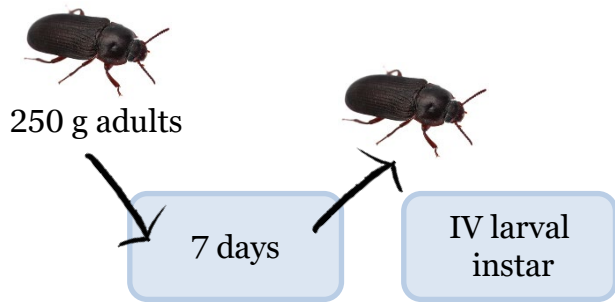
- Impact of **3** different **diets** on AMPs gene expression
- Impact of a selected diet on AMPs gene expression during larval conservation for ***in vivo* feeding trials**



Materials and Methods – insect rearing



Materials and Methods – insect rearing



70% wheat bran + 30% soy

T: $28 \pm 1^\circ\text{C}$, RH: $60 \pm 5\%$

Average weekly gain
Equal to or less than 50%

Wheat bran

Control diet

TM2

Wheat groats , wafer dough, vinasse , coffe
silvery film, breeding waste , feed waste

TM3

Wafer dough, breeding waste, feed waste,
Panettone

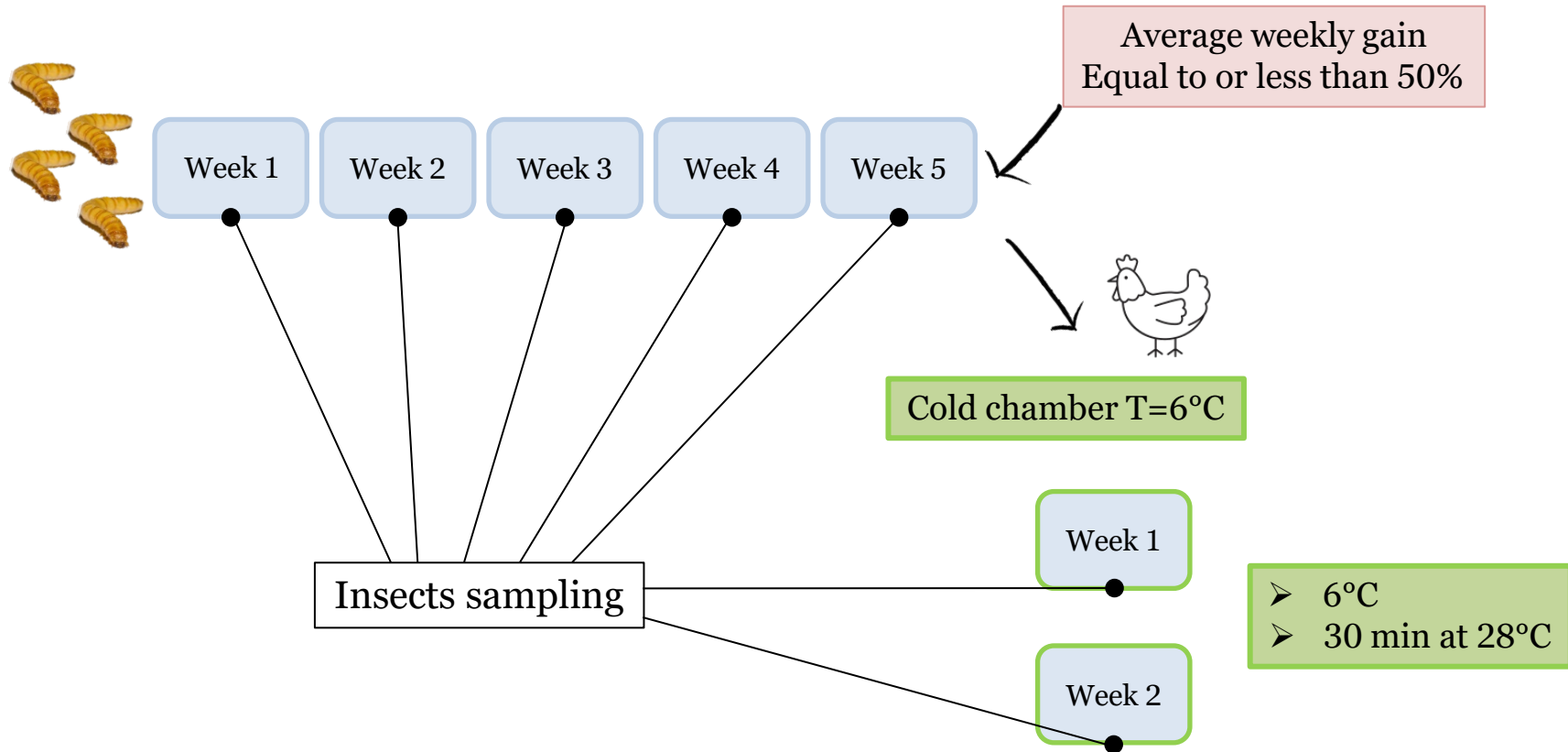
TM6

Rice husk, rice chaff, rice middlings,
breeding waste, feed waste

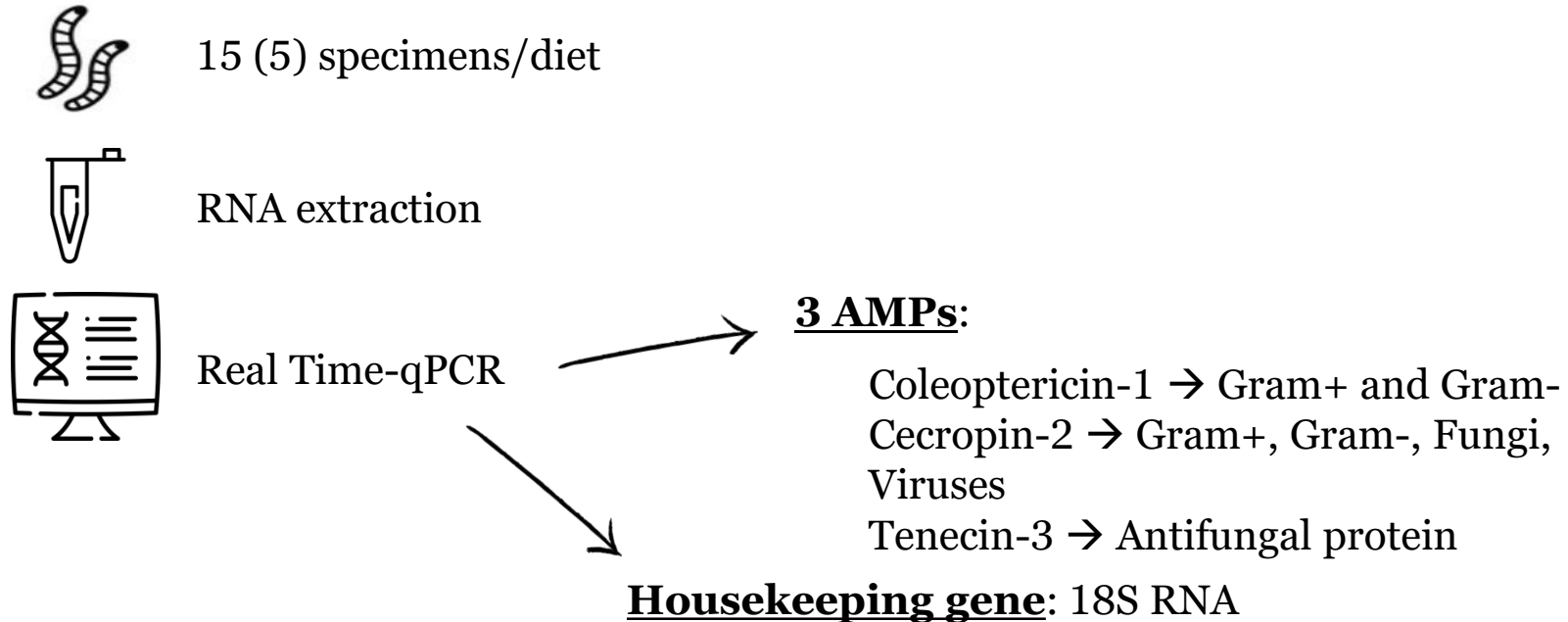
3 replicates/diet
10,000 larvae/replicate
Water agar 2%



Materials and Methods – insect rearing

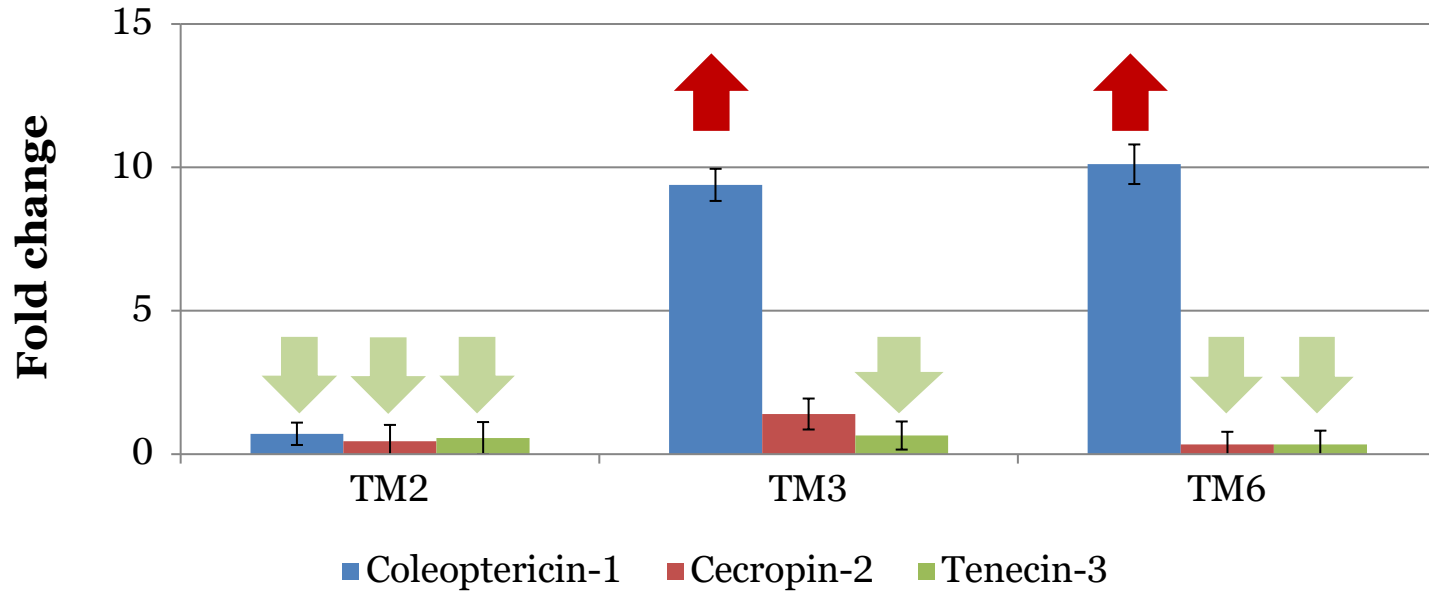


Materials and Methods - Expression of genes encoding for **AMPs**



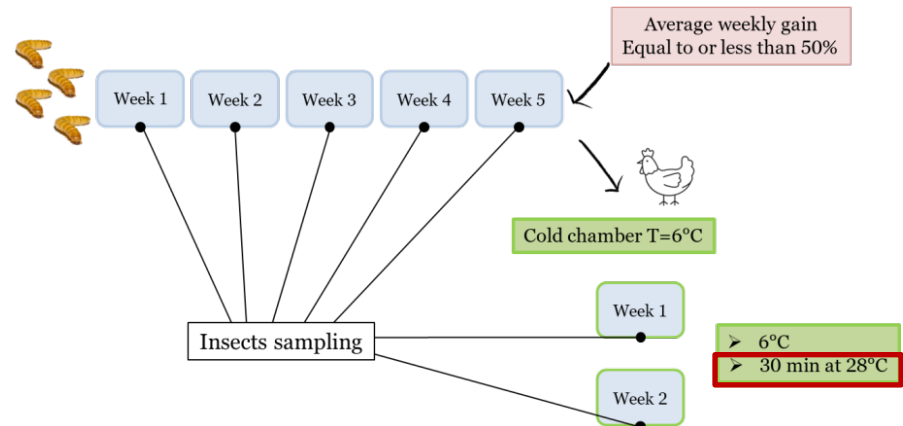
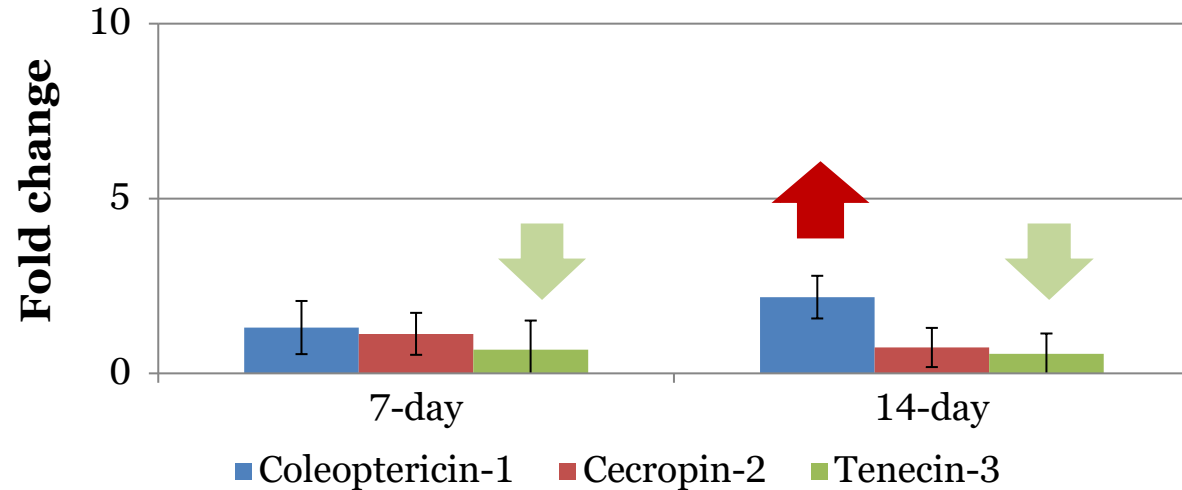
Zhu *et al.*, 2014. J. Asia. Pac. Entomol., 17(3): 339-342

Preliminary results



Preliminary results

TM6



Conclusion

- Modulation in the diet causes important changes in AMPs encoding-gene expression
 - TM2 negatively affects the gene expression of all tested AMPs
 - TM3 and TM6 → up-regulated the gene coding for Cecropin-1
→ negatively affects Cecropin-2 and Tenecin-3
- The timing of insect conservation in cold chamber influences AMPs gene expression
- Necessity to analyse also other AMPs and cellular responses

Other research projects



Funded by
the European Union
NextGenerationEU



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InProFarm, an Italian project to promote insect probiotic-assisted farming

The InProFarm project is funded by the European Union - Next Generation EU, National Recovery and Resilience Plan (NRRP) - Mission 4, Component 2, Investment 1.1, CALL PRIN 2022, Grant number 2022L4NJMK



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***Tenebrio molitor* hemolymph: a complex machinery which protects against pathogens**

The HeMiTool project is funded by the European Union – Next Generation EU, National Recovery and Resilience Plan (NRRP) - Mission 4, Component 2, Investment 1.1, CALL PRIN 2022 PNRR, Grant number P20228WWB7