

## Introduction

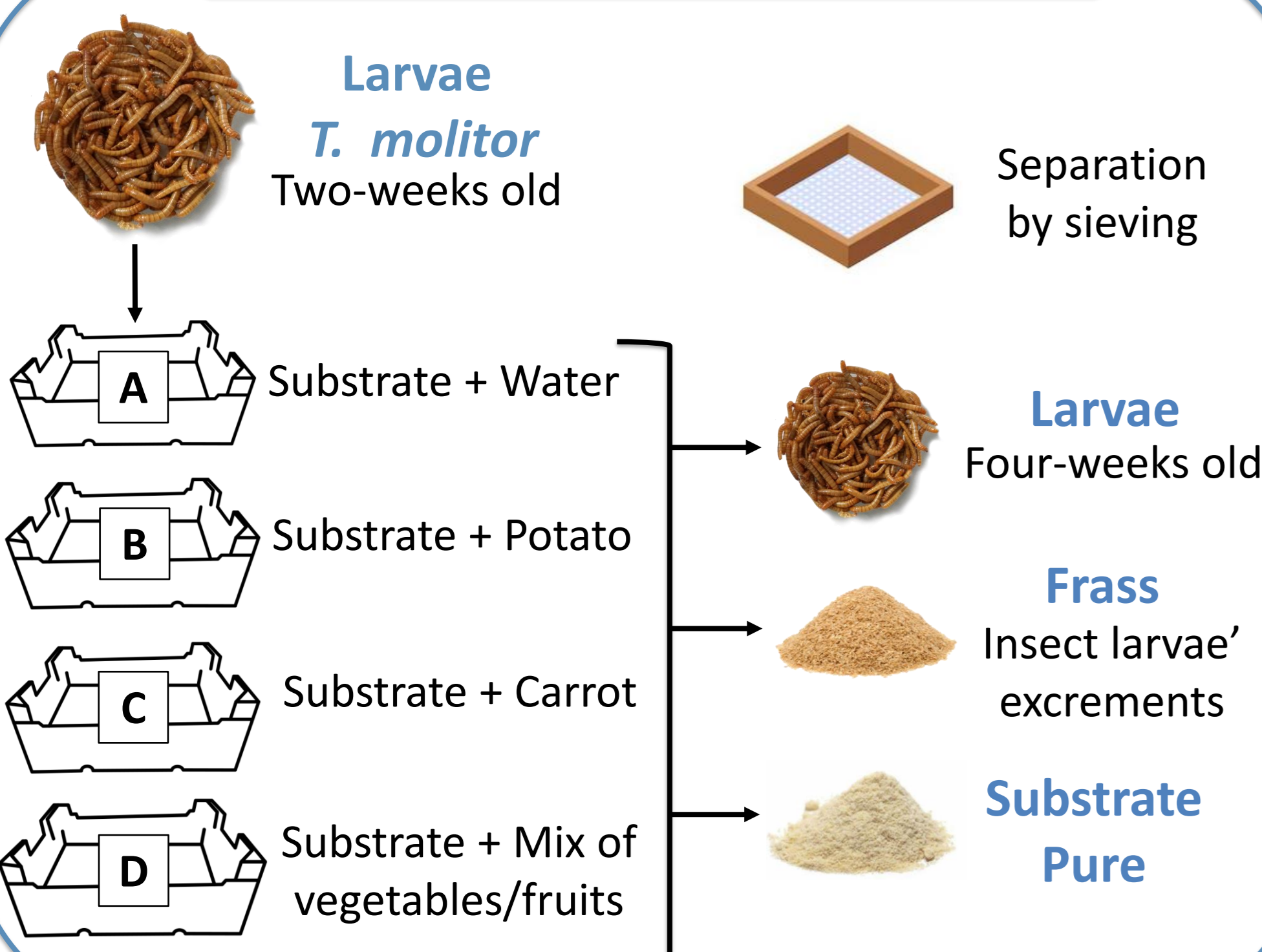
In a world facing an environmental crisis, the increase in world population coupled with a growing demand for natural resources emphasizes the importance of adopting new dietary patterns based on health and sustainability (1). The consumption of edible insects (e.g. *Tenebrio molitor*) offers an alternative to livestock consumption, providing a sustainable food production cycle that ensures food security with minimal environmental impact (2). One of the major challenges in the insect production industry is the selection of its feeding regime, since it has a direct impact on the growth performance and in nutritional profile of *T. molitor*, as well as on its safety, since it may lead to changes in the insect's microbiota and the presence of pathogenic microorganisms (3).

## Purpose

This study aimed to analyze the microbiology of *Tenebrio molitor* larvae subjected to different feeding regimes (substrate with water, potato, carrot, or a fresh vegetable mix).

## Methods

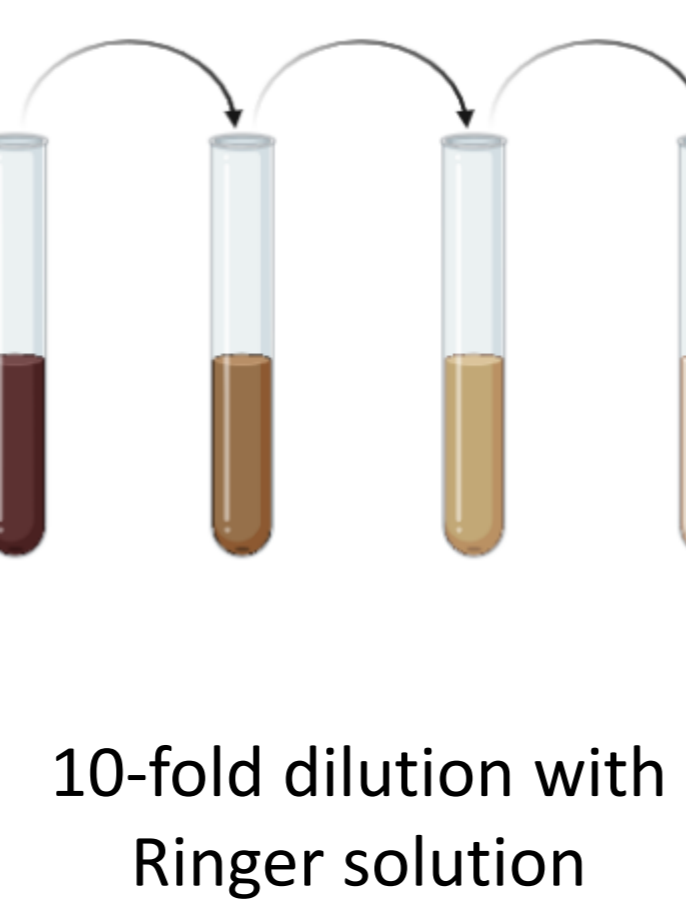
### 1. Sampling



### 2. Microbial count

#### Larvae, Frass and Substrate samples

10 g of sample + 90 ml BPW



- *Enterococcus* (Slanetz and Bartley, 37 °C, 48 h)
- *Bacillus cereus* (MYP, 30 °C, 24 h)
- Aerobic bacillus spores (70 °C, 10 min – BHI, 30 °C, 48 h)
- Molds and Yeasts (Rose Bengal, 25 °C, 5 days)
- Total viable counts at 30 °C (PCA, 30 °C, 72h)
- Lactic Acid Bacteria (MRS, 30 °C, 72 h)
- *Escherichia coli* (TBX, 44 °C, 18-24 h)
- *Enterobacteriaceae* (RAPID'Enterobacteriaceae, 37 °C, 24 h + 24 h)
- Anaerobic Bacillus spores (70 °C, 10 min – BHI, 30 °C, 48 h, anaerobic chamber)

### 3. Detection of *Salmonella* spp. and *Listeria* spp.

According to ISO 6579



Larvae, Frass and Substrate samples



According to ISO 1129

## Results

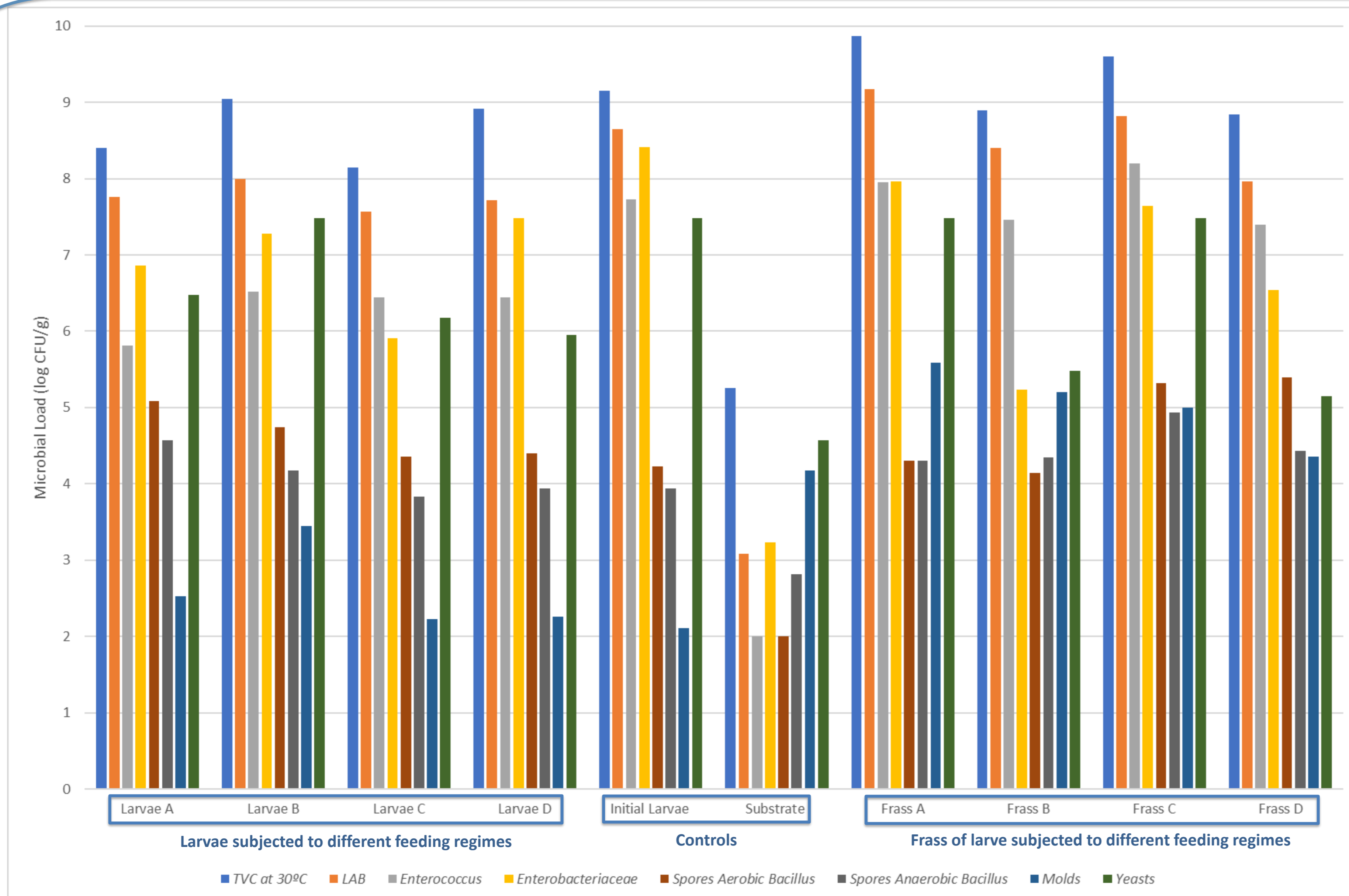


Figure 1. Microbiological analysis (log CFU/g) of *T. molitor* larvae submitted to different feeding regimes and the respective frass.

- In general, the original substrate had a lower microbial load, while initial larvae (two-weeks old) had a higher microbial load for TVC, LAB, *Enterococcus*, *Enterobacteriaceae* and yeasts.
- Larvae fed with potatoes had slightly higher microbial loads, especially for TVC, LAB, and *Enterobacteriaceae*. On the contrary larvae fed with carrot had the lower loads for TVC, LAB, aerobic and anaerobic bacillus spores and molds.
- *Escherichia coli* was only found at low levels in the substrate (1.0 log CFU/g) and *B. cereus* in the frass of larvae fed with potato (4.7 log CFU/g).
- The microbial load of the larvae and respective frass was compared. It was found that only in the potato substrate regime the microbial load was not higher in the frass.
- *Salmonella* spp. and *Listeria* spp. were not detected.

## Conclusion

- This preliminary study indicates apparent differences in the microbial loads of larvae fed with different regimes. An initial substrate with potato seems to increase the microbial load of both larvae and frass.
- Further studies of the microbial community using culture-independent methods are an alternative to expand knowledge of the microbiota of *T. molitor* and to overcome the detection limits of this technique.
- Although untreated larvae are not fit for human consumption, it is important to further analyze the effect of different treatments on the quality of the final product.

## References and Acknowledgements

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