Impact of different diets and starvation periods on microbial load of *Tenebrio molitor* larvae

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CIÊNCIA 2024 ENCONTRO COM A CIÊNCIA E A TECNOLOGIA EM PORTUGAL

Introduction

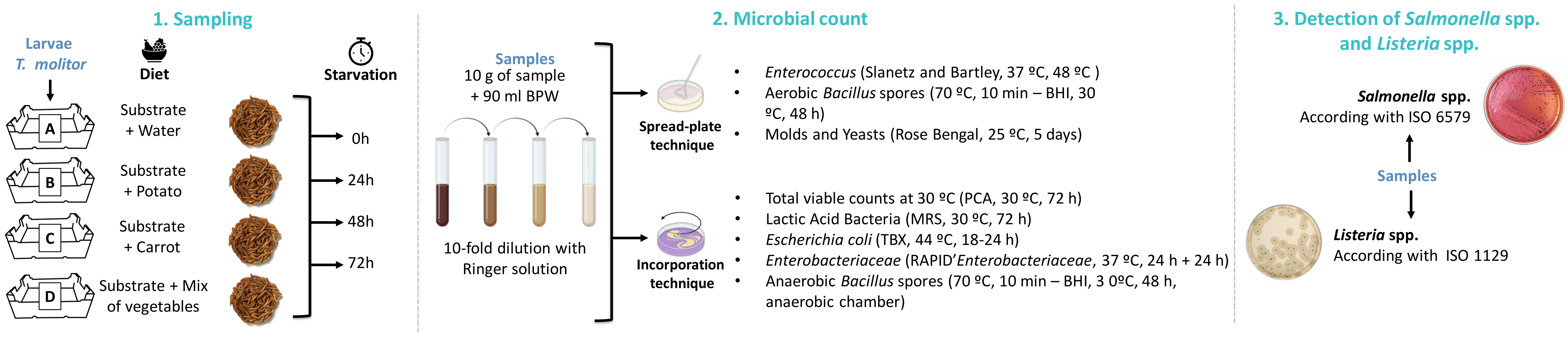
In a world facing an environmental crisis, the growth of the world's population coupled with an increasing demand for natural resources emphasizes the importance of adopting new dietary patterns based on health and sustainability (1). The consumption of edible insects (e.g. *Tenebrio molitor*) offers an alternative to animal protein sources, providing a sustainable food production cycle that ensures food security with minimal environmental impact (2).

The main challenges in the *T. molitor* production industry are the selection of an appropriate feeding regime, as this affects the growth performance, nutritional profile and microbiota of the insect, and the processing steps required to commercialize mealworm larvae for human consumption. These steps have a direct impact on safety by allowing the gut contents to be discarded, thereby reducing the microbiological load and the presence of pathogenic microorganisms (3, 4). Typically, mealworm larvae are reared in a dry, starchy substrate supplemented by a water source, such as slices of vegetables or fruit. After being reared under optimal growth conditions, the larvae are sifted and then subjected to a fasting period of at least 24 hours, as required by law. This fasting process allows the intestinal contents to be discarded and limits the microbiological load, with the fasting period being a legal requirement of at least 24 hours (3, 4).

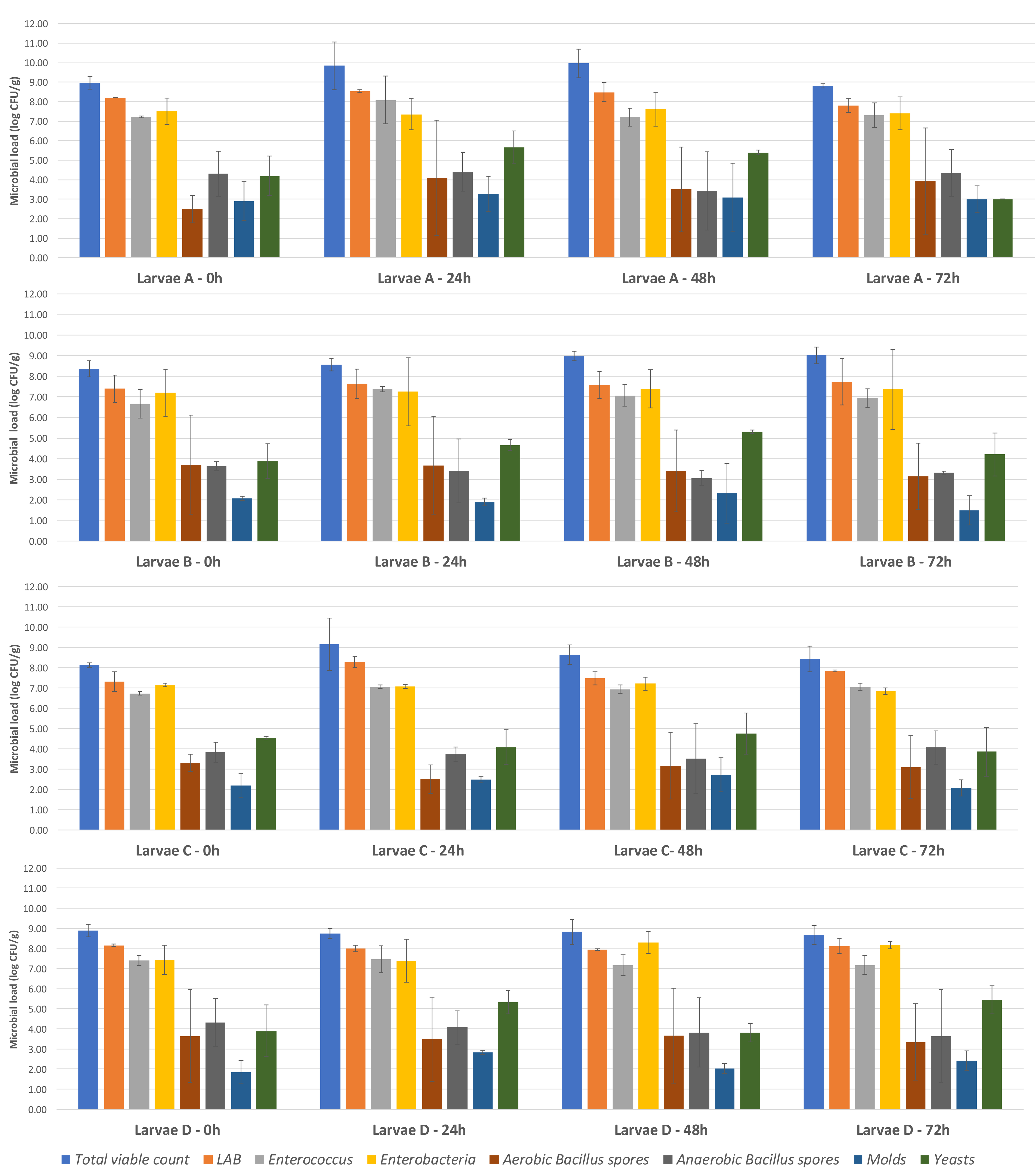
Objective

This study aimed to analyze the microbiology of *Tenebrio molitor* larvae subjected to various feeding regimes (substrate with water, potato, carrot, or a fresh vegetable mix) and subsequently subjected to different starvations periods (0 hours, 24 hours, 48 hours and 72 hours).

Methods



Results



Figures 1, 2, 3, 4. Microbiological analysis (log CFU/g) of *T. molitor* larvae subjected to four different feeding regimes: water (A), potato (B), carrot (C), mix of vegetables (D), respectively. Each group was subsequently submitted to four different starvation periods: 0 hours, 24 hours, 48 hours and 72 hours.

Discussion and conclusions

In general, the performance of starvation is dependent on the diet to which the larvae were subjected for each microbiological parameter evaluated.

A positive result is considered when, for larvae subjected to the same diet, the counts of a given parameter at a given starvation period (24 h, 48 h, 72 h) are lower than those observed in the control without starvation (0h). This was observed only for:

- **larvae A,** for anaerobic *Bacillus* spores after 48h, and for yeasts after 72h.
- **larvae B**, for aerobic *Bacillus* spores and anaerobic *Bacillus* spores after 24 h ,48 h and 72 h and for molds after 24 h and 72 h.
- **larvae C**, for *Enterobacteria* and yeasts after 24h and 72h; for aerobic *Bacillus* spores after 24h, 48h and 72h; and for anaerobic *Bacillus* spores after 24 h and 48 h.
- **larvae D**, for TVC, LAB and anaerobic *Bacillus* spores after 24, 48 h, 72 h; for *Enterococcus* after 48 h and 72 h; for *Enterococcus* after 48 h and 72 h; for aerobic *Bacillus* spores after 24 h and 72 h; and for yeasts after 4 8h.

Salmonella spp. and *Listeria* spp. were not detected in 10 g of each sample analysed.

In conclusion, these results call into question the efficacy of fasting periods in reducing the microbial load of *T. molitor* larvae, as previously reported in other studies, and highlight the importance of heat treatments following fasting to ensure the food safety of the final product.

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Acknowledgements

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project UIDB/50016/2020. Support for T. Carvalho was provided by a doctoral fellowship 2023.03709.BD (FCT).

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