ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELLA LOMBARDIA E DELL'EMILIA ROMAGNA
"BRUNO UBERTINI"
ENTE SANITARIO DI DIRITTO PUBBLICO

EAAP European Federation of Animal Science
Insect Study Commission

Poster Code: 28552

Black Soldier Fly larvae reared on contaminated substrate by *Listeria monocytogenes* and *Salmonella*

F. Defilippo
A. Grisendi
V. Listorti
M. Dottori
P. Bonilauri

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER) Sez. Reggio Emilia, Italy

BACKGROUND

Insects have been proposed as a high quality, efficient and sustainable alternative protein source. Using insects as a protein source can contribute to global food security via feed or as a direct food source for humans. In 2011, the world compound feed production has been estimated at 870 million tones and the turnover of global commercial feed manufacturing generated an estimated annual turnover and sales value equivalent to US$ 350 billion worldwide (http://www.ifif.org/). The UN Food and Agricultural Organization (FAO) estimates that the production of food worldwide will increase of about 70% by 2050. Concerning animal protein production, the International Feed Industry Federation (IFIF) believes that the production of meat (poultry/swine/beef) will even double. This poses severe challenges to the global capacity to provide enough feed. Insects could be an answer but the safety of protein from insects and subsequently the safety of meat and fish from animals fed on such a diet requires further assessment.

Black Soldier fly (*Hermetia illucens* L.) larvae (BSFL, Fig.1) have been noted to reduce the microbial load of substrates, decreasing bacteria concentrations in compost and fecal material. The purpose of our research was to investigate the capability of BSF larvae to reduce the concentration of *Salmonella* typhimurium and *Listeria monocytogenes* on an artificially contaminated substrate.

Fig.1 Black Soldier Fly (*Hermetia illucens*) larvae

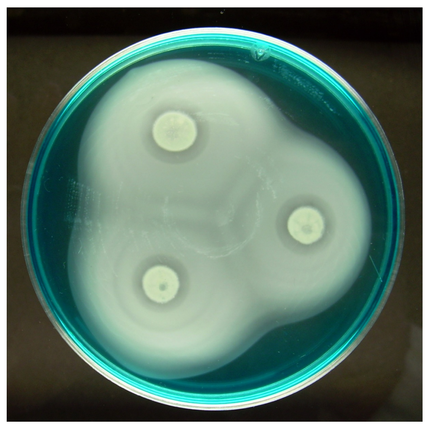
Fig.2 Black Soldier Fly (*Hermetia illucens*) pupae

MATERIALS AND METHODS

Two sets of experiments were conducted in order to investigate:

1) **the reduction of pathogens in contaminated substrate without larvae (control);**
2) **the behavior of challenged pathogens in larvae, pre-pupae, pupae and their substrates.**

BSF larvae: **BSFL were reared under controlled conditions (RH 70%, photoperiod 14:10 h (L:D) and temperature 25°C) on two substrates (Gainesville diet and a homemade artificial diet). The larval substrate was contaminated with S. typhimurium and *L. monocytogenes* (about 1x10^8 CFU g^{-1}). For each trial substrates and larvae were tested for pathogens enumeration, pH, and aw.**



Microbiological analysis

***Salmonella* Typhimurium detection (ISO 6579-1:2017) and enumeration (ISO 6579-2:2012)**

Samples was enriched (1:10) in Buffered Peptone Water for 18±2 h at 36°C ±2°C, 100 µl of sample was distributed on MSRV agar plate. Sample was incubated for 24/48 h at 41.5 °C ±1°C (Fig.3). The presence of *Salmonella* was confirmed by using Xylose-Lysine-Desoxycholate Agar and appropriate biochemical miniaturized tests. Micro MPN enumeration following part 2 of ISO 6579:2012 was applied. When the load of pathogens was above 3 log CFU/g, 10 fold dilution and direct plating on XLD and hektoen enteric agar was applied.

Listeria monocytogenes detection (ISO 11290-1:2017) and enumeration (ISO 11290-2:2017)

Samples was enriched in HF and plated in ALOA and OXFOD agar. Enumeration was performed by 10 fold dilution and direct plating on ALOA agar.

Fig.3 – MRSV agar Salmonella positive

RESULTS

In controls (contaminated substrate without larvae) the pH remained stable until the end of experiments (6.59-7.95) while aw remained stable until 7 days (0.977) but after this period aw decreased reaching 0.650. In control experiments the contamination of *L. monocytogenes* remain constant while a slow decline of *Salmonella* (-0.0077 log.conc/h D=130 h) was observed.

When larvae were present pH and aw remained stable through the duration of the study. *Salmonella* showed a *fast* decline in the first 8 days (-0.02 log.conc/h D=45 h) but after this initial reduction the pathogens remained constant in the substrate, showing a typical biphasic decline curve. While *L. monocytogenes* showed a very slow decline rate (0.0042 log.conc/h D=239 h) during the whole duration of the experiment.

In larvae *Salmonella* and *L. monocytogenes* showed the same concentration observed in the substrate, when larvae entered in pupal stage (fig.2) their contamination was significantly lesser than the substrate (-2 log).

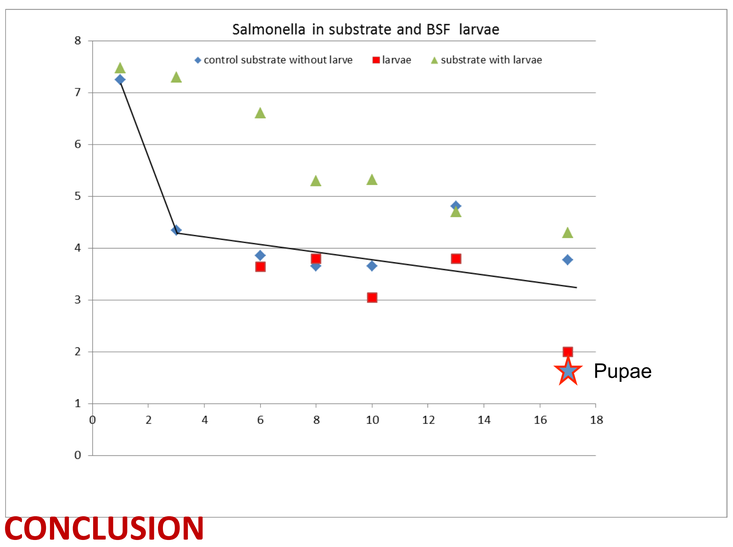
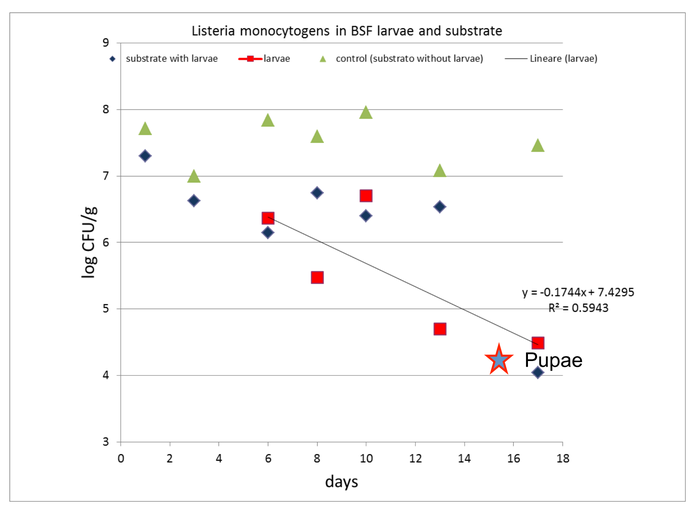


Fig.4: (left) Salmonella concentration (log CFU/g) in larvae, substrate and control (substrate without larva)

Fig.5: (right) Listeria monocytogenes concentration (log CFU/g) in larvae, substrate and control (substrate without larva)



CONCLUSION

This study confirm that larvae show the same pathogens concentration of their growing substrate and their feeding activity could reduce pathogens contamination in the growing substrate, but the rate of this action seems to be largely insufficient to reach the food and feed security objective.

References

[1] Newton, L., Sheppard, C., Watson, D.W., Burtle, G., Dove, R., 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. In: Report for Mike Williams, Director of the Animal and Poultry Waste Management Center. North Carolina State University

[2] Newton, G.L., Booram, C.V., Barker, R.W., Hale, O.M., 1977. Dried Hermetia illucens larvae meal as a supplement for swine. J. Anim. Sci. 44, 395–400.

[3] Harinder P.S. Makkara, Gilles Tranb, Valérie Heuzéb, Philippe Ankersa. 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197 (1–33)

Poster download

***Corresponding author: paolo.bonilauri@izsler.it – PRC2015005 -** E88C16000070001