

RICERCHE EFFETTUATE

IGIENE DEGLI ALIMENTI AD USO ZOOTECNICO

Defilippo° F, Grisendi° A, Listorti° V, Dottori° M, Bonilauri° P

Black soldier fly larvae reared on contaminated substrate by *L. monocytogenes* and *Salmonella*

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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) have been noted to reduce the microbial load of substrates, decreasing concentrations of bacteria in compost and faecal material. The purpose of our research was to study the behaviour of BSF larvae reared on diets artificially contaminated with *Salmonella typhimurium* and *Listeria monocytogenes*. Two sets of experiments were conducted in order to investigate: (1) the reduction of pathogens in contaminated substrate without larvae (control); (2) the presence of bacteria in larvae, prepupae, pupae and their substrate. 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 1 x10⁸ cfu/g). For each trial substrate and larvae were tested for pathogen enumeration, pH, and aw. In controls the pH remained stable until the end of experiments (6.59-7.95) while aw remained stable until 7 days (0.977) but decreases reaching 0.650 after this period. In substrates with larvae pH and aw remained stable through the duration of the study. The contamination of *L. monocytogenes* remained constant in control experiments while a slow decline of *Salmonella* (-0.0077 log.conc/h D=130 h) was observed. When larvae were present, *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 substrates, while *L. monocytogenes* showed a very slow decline rate (0.0042 log.conc/h D=239 h). When larvae enter in pupal stage their contamination with *Salmonella* and *L. monocytogenes* was significantly lesser than the substrate (-2 log). This study confirms 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 their action seem to be largely insufficient to reach the food and feed security objective.

Defilippo° F, Grisendi° A, Listorti° V, Fedrizzi° G, Dottori° M, Bonilauri° P

Bioaccumulation factor of cadmium in the different life stages the Black Soldier Fly, *Hermetia illucens*

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There is an urgent need to increase the supply of sustainable protein sources to be used in animal feed and the use of insect's protein provides a potential alternative to protein crops and fishmeal. For instance, fly larvae contains high levels of digestible protein with key amino acids, comparable with those found in high value protein sources such as soybeans, and could be recommended for use in animal feed. Among the insect species, there is an increasing interest in rearing black soldier fly larvae (BSF, *Hermetia illucens*). Larvae of this species can be used for biodegradation of organic waste and are a promising feed source for animal nutrition, being rich in protein. However, studies investigating safety aspects of the use of black soldier fly as feed are scarce. The aim of this study

was to investigate the potential bioaccumulation of cadmium from the feeding media to the larvae, pupae and adults of BSF. Two sets of experiments were conducted in order to investigate: Bioaccumulation Factor (BAF - metal concentration in the body divided by metal concentration in the feeding media) in BSF reared on substrate contaminated in all larval stages; BAF in BSF reared on contaminated substrate until the 3rd larval instar and transferred subsequently on control diet. BSFL were reared under controlled conditions (RH 70%, photoperiod 14:10 h (L:D) and temperature of 25°C) on two substrates: Gainesville diet for the control group and a cadmium contaminated diet, enriched with horse liver containing naturally high concentration of cadmium (1.303 mg/Kg), for the contaminated groups. In all experiments, larvae, prepupae, pupae and adults accumulated cadmium. The BAF of cadmium in larvae was between 3.98 and 2.33, in prepupae 3.54 and in pupae 2.39. Heavy metal BAF in adults was lower than in other life stages (1.25). In the 2'd experiment where BSFL were reared on contaminated substrate until the 3rd larval instar and then transferred on control diet, the BAF was much higher than the levels found in experiment 1 (4th larvae instar 11.27, prepupae 8.08 and pupae 6.19). So that, the Cd accumulated by larvae until the reaching of the 3rd larval instar is slowly eliminated by the larvae even though transferred on clean control diet. Moreover the variations of protein source in control diet (liver vs alfa alfa) has stretched the development time of the BSF in the experiment 2 and therefore the feeding time for each life stage in the experiment 2 was much longer than in the experiment 1, causing a further accumulation of the cadmium naturally present in control diet. Development time from hatching of the larva to the prepupal, pupal and adult stage generally increased with the increasing of heavy metal concentration in the feeding media. Our study reveal an high capability of BSF larvae and prepupae to accumulate cadmium and this could potentially limit their use for animal feed production. A broader understanding of the occurrence of this undesirable substance in processed larvae products is needed to assess feed and food safety.