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Starch digestion in *H. illucens* conversion: exploring the role of amylases from larvae and substrate

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The capacity of black soldier fly larvae (BSFL; *Hermetia illucens*) to transform organic substrates into body proteins and lipids suitable for animal nutrition is receiving growing attention. Carbohydrate content of BSFL diet is positively related to larval growth, fat body content and adult egg production, but underlying mechanisms of feed conversion remain to be explained. Previous studies reported amylase activity in larval midgut, and it has recently been shown that BSFL were highly efficient at digesting starch. This study focused on the effect of starch content and type on BSFL amylase activity and starch Estimated Digestibility (ED). BSFL were fed on five plant-based diets with different starch contents and types, and larvae and substrates were sampled after 4, 7 and 11 days of feeding, along with initial BSFL and diets. Each sample was ground in phosphate-buffered saline with protease inhibitor and centrifuged to collect water phase. Amylase activity was assessed using the Bernfeld technique and reported to total soluble protein measured according to Bradford. For substrate samples that could contain enzymes from the plant material, microbes or larvae, BSFL amylases were detected by Western-Blot using antibodies specific to insect amylases. This approach offers insight into larval amylase regulation mechanisms and the role of extra-oral digestion of starch in BSFL conversion systems.

Investigating the nutritional requirements of black soldier fly larvae using artificial substrates

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Due to the rising need for alternative protein, the black soldier fly gained interest of researchers worldwide. One reason for this is the insect's remarkable efficiency at converting feed into larval biomass, rich in high-quality protein. Moreover, they are able to grow on a high variety of organic side- and waste streams, allowing local production of high-quality protein, while also valorising organic waste. Even though their remarkable plasticity towards growing on a wide range of organic substrates has been proven in many studies, optimization is still necessary to increase the sector's viability. In order to optimize the sector, one important approach is to tailor the nutritional requirements of black soldier fly larvae. By doing so, models can be developed that predict larval growth based on substrate nutrient compositions, which can help combine different organic side-streams to create a more nutritionally complete substrate for black soldier fly rearing. Therefore in this study, multiple designs were developed and tested to study the effects of macronutrient contents on the growth of black soldier fly larvae through the use of artificial substrates. Substrates were composed of casein, sunflower oil, potato starch and cellulose, which allowed precise formulation of substrates, and could reduce the background noise such as the presence of complex sugars and unbalances in amino acid profiles. In this study, a model was built, showing significant main, interaction and quadratic effects of substrate protein and fat contents. A maximal larval growth was acquired at a substrate protein content of 30.12% and a fat content of 8.75%. The effect of carbohydrates on larval growth was not significant in this study, however this could be due to poor digestibility of raw potato starch by the larvae. While larvae tend to grow best on high-protein substrates, research shows that the protein conversion efficiency is inversely proportional to substrate protein content, meaning we may need to reconsider our approach to substrate optimization if we want to balance between maximizing larval growth and improving protein conversion efficiency.