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Short Communication

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Does Consumption of Baker's Yeast (*Saccharomyces cerevisiae*) by Black Soldier Fly (Diptera: Stratiomyidae) Larvae Affect Their Fatty Acid Composition?

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Abstract

Fatty acids are important compounds for insects, but the requirements for essential fatty acids may differ between insect species. Most of the fatty acids are acquired through the insect's diet; therefore, supplementing the diet with baker's yeast (*Saccharomyces cerevisiae* Meyen *ex* E.C. Hansen), which produces unsaturated fatty acids, was predicted to affect the fatty acid composition of the insect. The tested insect was the black soldier fly (BSF) (*Hermetia illucens* L.), that is used as a source of protein and fat in feed. Therefore, there is importance for BSF larvae (BSFL) nutritional composition, especially the unsaturated fatty acids content, which is one of the nutritional limitations for mammalian diets. The dominant fatty acids of the tested BSFL were the saturated fatty acids: lauric, myristic, and palmitic acids, as found in other BSF studies. Oleic acid (c18:1) and linoleic acid (C18:2) were the abundant unsaturated fatty acids in the BSFL. The proportion of linoleic acid was higher in the substrate with the supplemental yeast; however, this did not affect its proportion in the larvae. The higher proportion of linoleic acid may have been exploited as a source for production of saturated lauric acid. Therefore, providing unsaturated fatty acids to the substrate through supplemental baker's yeast is not the most efficient way to increase the proportion of unsaturated fatty acids in the larvae.

Key words: unsaturated fatty acid, insect–yeast interaction, BSF, insects for feed

Fatty acids are important compounds for insects. They have a role in energy storage, cell, and cuticle structure and production of pheromones (Stanley-Samuelson et al. 1988). The major fatty acid groups in insects are associated with triacylglycerol as a source of metabolic energy, and phospholipids and sterol ester components for cellular and subcellular membranes (Stanley-Samuelson et al. 1988). The requirements of essential fatty acids may differ between insect species and most of the fatty acids are acquired through the insect's diet (Canavoso et al. 2002). Therefore, the diet content can change the fatty acid composition of the insect and its life history traits (Barroso et al. 2017, Lehtovaara et al. 2017, Rutaro et al. 2018). As the interest in insects for food and feed is increasing (van Huis 2020), there is importance in manipulating the fatty acid content of the insects, especially increasing the unsaturated fatty acids, which are one of the nutritional limitations for mammalian diets (Anderson et al. 2009)

The insect model used in this study was the black soldier fly (BSF) (Diptera: Stratiomyidae; *Hermetia illucens* L.), native to the tropical and sub-tropical regions of America. The BSF is one of the insects most commonly used for mass production (mainly for feed) (Makkar et al. 2014). The larvae are used as a decomposer of organic waste and as feed due to their high concentration of proteins (42%) and lipids (30%) in the V instar larvae and prepupae stages (Gianetto et al. 2020), which composed mainly from the saturated lauric acid (C12:0). The unsaturated fatty acid content of the BSF can be increased, to some degree, by the composition of its diet (Barroso et al. 2017, Oonincx et al. 2020).

Due to their detritivorous nature, BSF larvae (BSFL) thrive in environments of decaying organic materials, which abound in yeast, yeast like fungi, and other microorganisms. Yeast, which is present in the substrate, also affects the microbial composition of the larval gut (Boccazzi et al. 2017). Interactions between yeast and insects are known from various insect orders (Vega and Blackwell 2005). It has been suggested that yeast provide insects with organic nitrogen, essential vitamins and lipids and allow food localization, while the insect provides the yeast with a mode of dispersal or a protected environment for reproduction (Stefanini 2018). However,

the mechanism of the interactions between yeast and BSFL is not known. As yeast are known to produce fatty acids (Tehlivets et al. 2007), we hypothesized that the presence of yeast in the feeding substrate of the BSFL will affect the fatty acid composition of the larvae due to direct feeding or symbiotic metabolic interactions. To test his hypothesis, we supplied baker's yeast (*Saccharomyces cerevisiae* Meyen *ex* E.C. Hansen) to the feeding substrate, as baker's yeast is known to produce the unsaturated oleic acid (C18:1 *cis*-9) (Uemura 2012). Therefore, we tested its effect on the presence of yeast in the larval gut, the fatty acid composition of the insect and the expression of genes related to fatty acid metabolism.

Materials and Methods

Larval Feeding Experiment

Black soldier fly eggs were purchased from freezeM (Israel). The eggs were divided into two diets, which were based on apples (55% of weight) and beer malt (15%) (based on previous experience). The difference between the diets was their protein source (which is a limiting factor in the BSFL diet): 1) 50 ml yeast liquid from beer brewery waste (50% yeast, Bazelet Brewery, Israel)—diet with supplemental yeasts; 2) 8% casein (Sigma) added with 30 ml water—diet with naturally occurring yeast and with protein level that resemble the 'supplementary yeast diet'; 3) 1,000 larvae were placed in 2-liter cages, with five replicates for each diet. The diet was provided twice a week to the larvae according to larval development. The experiment was conducted in a rearing chamber (30°C, 30% RH). The larvae were harvested after 50% of the larvae reached the prepupal stage, which was identified by the change in color from cream to black.

Quantifying Yeast Abundance in the Larval Gut

To test whether the treatments affected yeast abundance in the larval gut, we quantified the yeast using qPCR. The larvae were dissected and the DNA was extracted from the whole gut using DNeasy Blood & Tissue kit (Qiagen). A qPCR was conducted with general primers for yeast (YEASTR and YEASTF (Hierro et al. 2006) targeting the D1/D2 domains of the 26S rRNA gene. The qPCR reactions contained 10 µl of HY-SYBR (hy-labs, Israel), 2. 0 µl (10 µM) of each of the forward and reverse primers, and 3. 0 µl of purified DNA. The final volume was adjusted to 20 µl using sterilized H_2O. Amplification conditions were 95°C for 3 min, followed by 45 cycles of 95°C for 10 s, 59°C for 10 s, 72°C for 10 s, with a final extension at 72°C for 180 s. Three technical replicates of each sample were amplified using the CFX384Touch Real-Time PCR Detection System (BioRad). The CFU/ml concentrations of each sample were evaluated by comparing the threshold value (Cq) with the Cq value of a standard curve of various concentrations of one of the samples (with supplemental yeast).

Lipid Extraction and Fatty Acid Analysis

Lipids were extracted from the larvae and the substrate using the Sohxlet method with hexane (Hara and Radin 1978). Benzophenone was added as an internal standard. After hydrolization of the fatty acids with S_2SO_4, the fatty acid methyl esters (FAME) were analyzed by gas–liquid chromatography (7890A, Agilent Technologies) using a Zebron ZB-FAME column (30 m × 0.25 mm × 0.20 µm; Phenomenex, USA) under the following conditions: injector: 240°C; detector: 260°C; H_2 as carrier gas; temperature program: 140°C for 2 min, followed by an increase of 10°C/min to 190°C, then 3°C/min to 260°C. Peaks were identified by comparing their retention times with those of the corresponding standards (Supelco 37 Component FAME Mix, Sigma).

Analysis of Gene Expression Related to Fatty Acid Metabolism

Total RNA was extracted from the BSFL fat bodies using an RNeasy kit (Qiagen). cDNA was synthesized using a Verso cDNA Synthesis Kit (Thermo Fisher Scientific). Transcript levels of the acyl-CoA dehydrogenase gene were related to fatty acid metabolism (*fas*) with the primers *fas*R and *fas*F (Giannetto et al. 2020). The RT–PCR reactions contained 10 µl of HY-SYBR (hy-labs, Israel), 2.5 µl (10 µM) of each of the forward and reverse primers, and 2.0 µl of purified cDNA. The final volume was adjusted to 20 µl using sterilized H_2O. Amplification conditions were 95°C for 3 min, followed by 45 cycles of 95°C for 30 s, 59°C for 60 s, 72°C for 30 s with a final extension at 72°C for 180 s. Four technical replicates of each sample were amplified using the CFX384Touch Real-Time PCR Detection System (BioRad). The CFU/ml concentrations of the samples were evaluated by comparing their threshold values (Cq) with the Cq value of a standard curve of various concentrations of one of the samples (with supplemental yeast).

Data Analysis

Differences in yeast abundance in the larval gut, fatty acid composition, and gene transcription levels between the different treatments and life stages were analyzed using two-way ANOVA. All statistical analyses were conducted using R software (Team and R Core Team 2012).

Results

Quantifying Yeast Abundance in the Larval Gut

Yeast abundance was higher (by an order of magnitude) in the gut of stage V larvae compared to prepupal larvae ($F_{df=1,55} = 3.55$, $P = 0.05$), with no significant difference between treatments ($F_{df=1,55} = 0.41$, $P = 0.52$; Fig. 1).

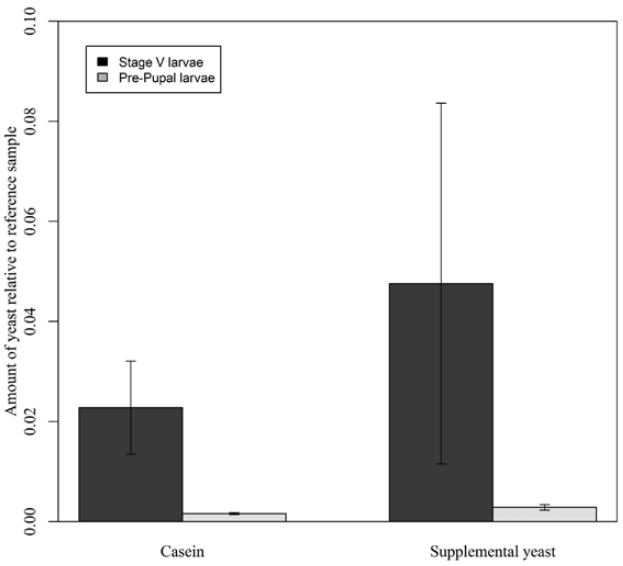

Fig. 1. Amount of yeast (relative to reference sample) in the BSFL gut reared on a substrate with casein or supplemental yeast (*S. cerevisiae*) as a protein source. Black bars represent stage V larvae, gray bars represent prepupal larvae. Error bars represent standard error.

Lipid Content and Fatty Acid Composition

Total lipid content of the larvae was not affected by diet or life stage (Life stage: $F_{df=1,15} = 0.72$, $P = 0.41$, Diet: $F_{df=1,15} = 0.47$, $P = 0.37$; Fig. 2A). Similarly, total lipid content of the substrate was not affected by diet ($F_{df=1,3} = 2.24$, $P = 0.23$; Fig. 2B). Differences in fatty acid composition in the larvae were found for saturated lauric acid (C12:0), which comprised a higher proportion in the prepupal larvae ($F_{df=1,19} = 6.58$, $P = 0.02$), and palmitic acid (C16:0), which comprised a higher proportion in the stage V larvae ($F_{df=1,19} = 4.1$, $P = 0.05$). Unsaturated oleic acid (C18:1 *cis*-9) comprised a higher proportion in the stage V larvae ($F_{df=1,19} = 7.32$, $P = 0.01$) and linoleic acid (C18:2 *cis*-9,12) comprised a higher proportion in the larvae fed with casein ($F_{df=1,19} = 6.2$, $P = 0.02$; Fig. 3). Differences in fatty acid composition of the substrate were found for lauric acid (C12:0) and linolelaidic acid (C18:2 *trans*-9,12), which comprised a higher proportion in the substrate with supplemental yeast ($F_{df=1,8} = 7.57$, $P = 0.02$ and $F_{df=1,8} = 4.75$, $P = 0.05$; respectively), and for palmitic acid (C16:0), which comprised a higher proportion in the substrate with casein ($F_{df=1,8} = 4.54$, $P = 0.05$; Fig. 4).

Gene Expression

The expression of acyl CoA dehydrogenase (*fas*) was higher in the prepupal larvae and in the supplemental yeast treatment (treatment: $F_{df=1,15} = 7.02$, $P = 0.02$; stage: $F_{df=1,15} = 16.76$, $P < 0.001$; Fig. 5).

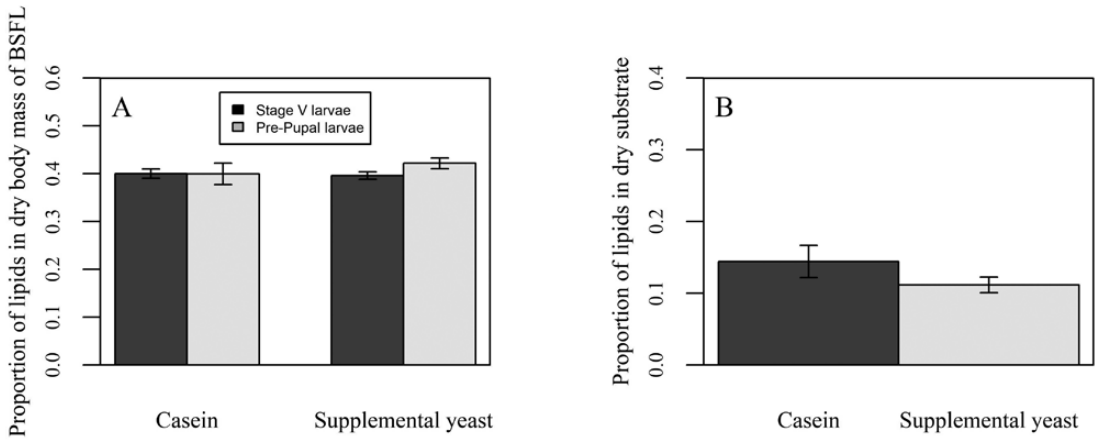

Fig. 2. Proportion of total lipids in BSFL dry matter (A) and in the feeding substrate (B), where the feeding substrate included casein or supplemental yeast (*S. cerevisiae*) as a protein source. In (A), black bars represent stage V larvae, gray bars represent prepupal larvae. In (B), black bars represent the substrate with casein and gray bars represent the substrate with supplemental yeast. Error bars represent standard error.

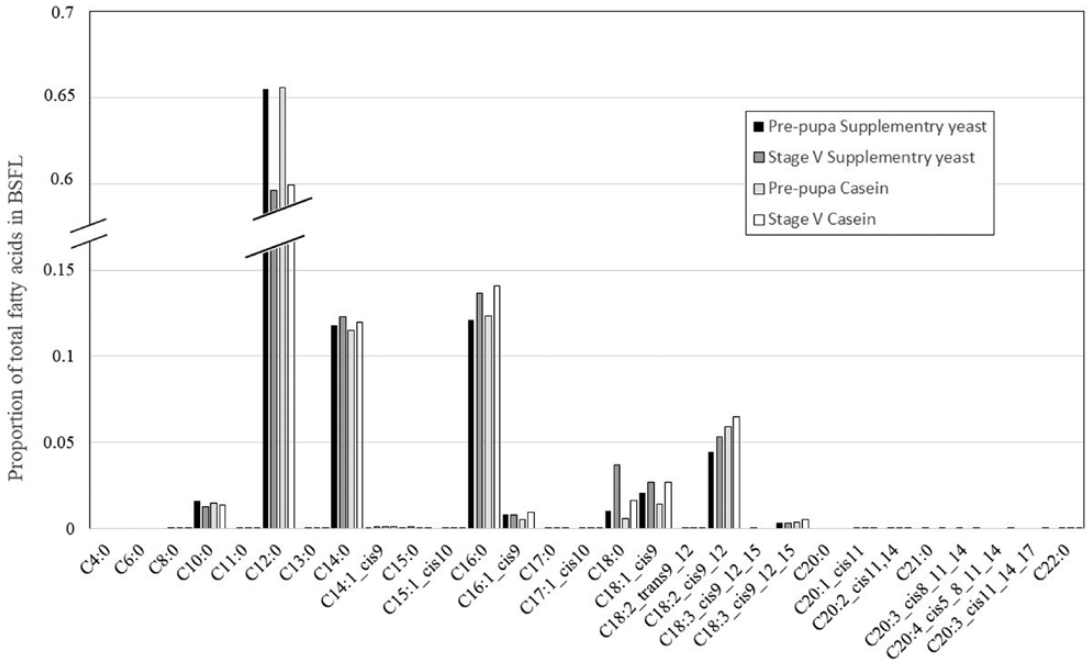

Fig. 3. Proportion of the different fatty acids in BSFL. Black and dark gray bars represent prepupal and stage V larvae (respectively) fed with supplemental yeast (*S. cerevisiae*) as a protein source. Light gray and white bars represent prepupal and stage V larvae (respectively) fed with casein as a protein source. Error bars represent standard error.

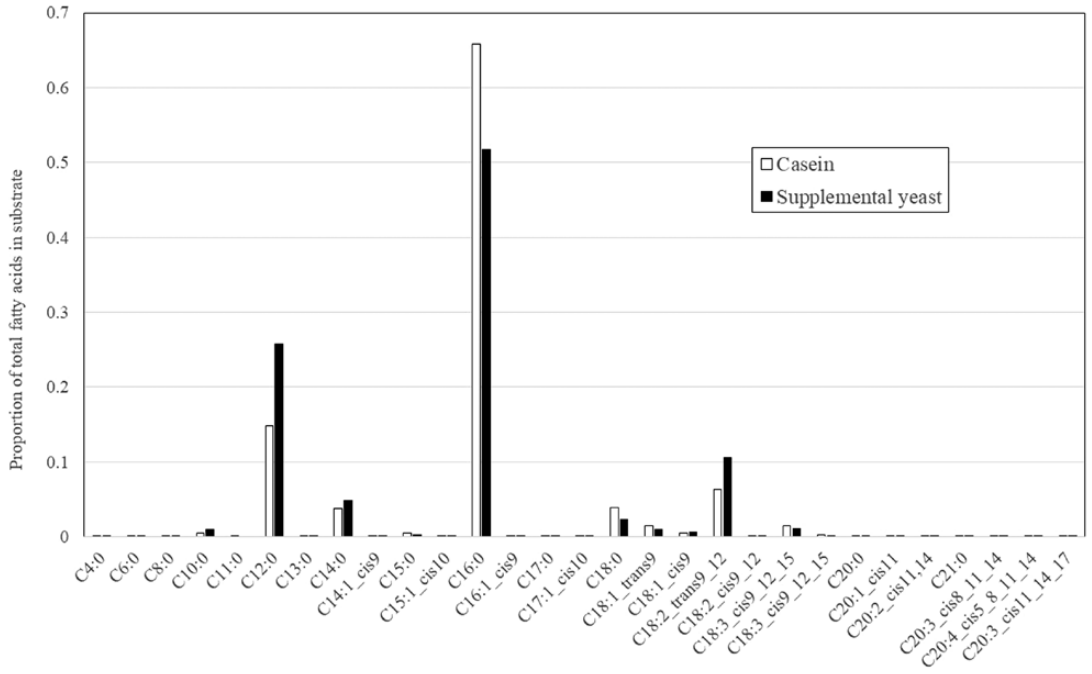

Fig. 4. Proportion of the different fatty acids in the feeding substrate of the BSFL. White bars represent the diet with casein as a protein source. Black bars represent the diet with supplemental yeast (*S. cerevisiae*) as a protein source. Error bars represent standard error.

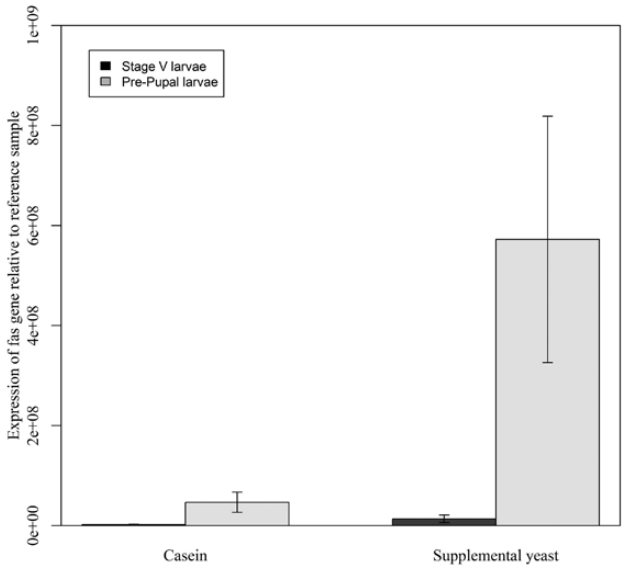

Fig. 5. RNA expression of *fas* gene in BSFL fat bodies (relative to reference sample). Black bars represent stage V larvae, gray bars represent prepupal larvae. Error bars represent standard error.

Discussion

This study tested the effect of supplemental yeast, *S. cerevisiae*, in the feeding substrate of BSF larvae on the fatty acid composition of the larvae. The yeast abundance did not differ when yeast were added to the substrate. It could be that the abundance of different yeast species originating in the substrate was high, as known from other studies with brewery waste (Gonzalez Pereyra et al. 2011), but their identity varied between the two treatments. The yeast composition of these two treatments will be analyzed in further studies.

The dominant fatty acids of the BSF were the saturated fatty acids: lauric, myristic, and palmitic acids, as found in other studies (Giannetto et al. 2020, Smets et al. 2020, Surendra et al. 2016). Lauric and myristic acids are known to be produced de novo by the larvae (Hoc et al. 2020), and the low levels of these acids in the substrate, compared to their proportion in the larvae, emphasize this. The higher levels of lauric acid in the substrate with the supplemental yeast did not affect the proportion of this acid in the larvae. However, lauric acid comprised a higher proportion in the prepupal stage in both treatments. These results suggest that lauric acid is the preferred fatty acid for accumulation in the larvae, especially toward the pupal stage. Palmitic acid comprised a higher proportion in the substrate, particularly the one with casein, however these differences did not affected the proportion of this fatty acid in the larvae. Palmitic acid comprised a higher proportion in the stage V larvae; it could be that this fatty acid is being accumulated from the substrate (Hoc et al. 2020) and used for the metabolism of lauric acid. This may explain the increase in the transcription of the *fas* gene in the prepupal stage; however, surprisingly an increase was also observed in the treatment with supplemental yeast.

Oleic acid (c18:1) and linoleic acid (C18:2) were the abundant unsaturated fatty acids in the BSFL. Although previous studies have shown that oleic acid is more abundant than linoleic acid (Giannetto et al. 2020, Hoc et al. 2020), our study showed contrasting results. Linoleic acid comprised a higher proportion in the substrate with supplemental yeast, although *S. cerivisiae* is known to oxidate linoleic acid rather than to produce it (Van Roermund et al. 2003). Moreover, the higher linoleic acid proportion in the substrate did not affect its composition in the larvae. The higher proportion of linoleic acid may have been used as a source for production of saturated lauric acid. Therefore, feeding the BSFL with extensive unsaturated fatty acids will result in an increase in lauric acid, although other studies have shown that feeding BSFL with flaxseed and the microalga, *Schizochytrium*, which are rich in unsaturated omega 3 fatty acids (linolenic acid C18:3

and eicosapentaenoic acid C20:5, respectively), increases the accumulation of these fatty acids in the larvae (El-Dakar et al. 2020, Oonincx et al. 2020). It may be that the metabolic fate of polyunsaturated fatty acids such as omega 3 is different and they are not being metabolized to shorter saturated fatty acids. It seems that although yeast increased the proportion of linoleic acid (C18:2 *cis*-9,12) in the substrate, surprisingly, the proportion of this fatty acid was similar in the larvae from both treatments, suggesting that this fatty acid is nor absorbed or accumulated in the BSF larvae. Further studies should test the accumulation of other unsaturated fatty acids provided through the BSF diet.

To conclude, as the demand for unsaturated fatty acids increase, producing BSF larvae that are rich in such fatty acids through supplemental baker's yeast in the insect's diet, is not the most efficient way. Further research must be done to examine the interactions of BSFL with specific yeasts that are adapted to the larvae and can provide unsaturated fatty acids that will be accumulated by them.

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Author Contributions

I.O.: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing—original draft; Writing—review and editing. T.V.: Data curation; Investigation; Methodology; Writing—review and editing. A.J.L.: Methodology; Writing—review and editing. R.G.: Supervision, Writing—review and editing.

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