RESEARCH ARTICLE

An investigative study to utilise the *Fusarium*-damaged wheat as a feedstock for the black soldier fly larvae (*Hermetia Illucens*).

V. Kumar[1], C. Ochoa-Sanabria[2], T. Tanaka[1*]

[1]Department of Food and Bioproduct Sciences, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Dr., Saskatoon, Saskatchewan S7N5A8, Canada

[2]Enterra Feed Corporation, Maple Ridge, British Columbia V2X 0T4, Canada

[*]Corresponding author's email: takuji.tanaka@usask.ca

Abstract

This study investigated *Fusarium*-damaged wheat kernels (FDK) as a potential feeding substrate for black soldier fly larvae (*Hermetia illucens*; BSFL). *Fusarium*-damaged kernels are considered unsuitable for food and feed due to the presence of mycotoxins. Mycotoxins, like deoxynivalenol (DON), pose health risks when consumed by animals at concentrations exceeding the limits established by the Canadian Food Inspection Agency. However, BSFL have shown higher tolerance to mycotoxins, suggesting that FDK may be used as a feeding substrate for BSFL intended for animal consumption. To assess this, three FDK-based diets with varying concentrations of DON (4.49 ± 0.08 ppm, 6.04 ± 0.02 ppm, and 6.83 ± 0.04 ppm) and a healthy wheat-based diet (0 ppm DON; control diet) were formulated to grow the larvae. The diets were fed to larvae to assess their preference based on DON concentration. Concurrently, the accumulation of DON in BSFL biomass and its effects on growth parameters were evaluated. The larvae showed no preference for any DON concentration. The DON levels accumulated in the BSFL biomass were minimal, regardless of the DON concentration in the feed ($p<0.05$), with the highest recorded at 0.87 ± 0.04 ppm compared to 6.83 ± 0.06 ppm in the diet. Despite the potential harm of FDK to animals, the growth parameters of BSFL improved, with larvae on FDK-based diets reaching a live body weight of 185.0 ± 3.2 mg compared to 177.6 ± 4.2 mg for the control on Day 15. The nutritional profile remained nearly identical across all DON concentrations (~41% crude lipid and ~39% crude protein in dried biomass). These findings suggest that BSFL raised on FDK-based diets can be used effectively for feed purposes.

Keywords: Insect protein, fusarium head blight, deoxynivalenol, mycotoxin

Declaration of competing interests

The authors do not have any conflict of interest.

1 Introduction

Fusarium head blight (FHB), also known as scab, is a fungal disease that affects wheat (including durum), barley, oats, and other small cereal grains globally (Mielniczuk & Skwaryło-Bednarz, 2020). The fungal toxin (mycotoxin) produced by *Fusarium*-infected seeds can contaminate the grain, making it unconsumable to animals. Moreover, fungal development in the grain disrupts kernel development, lowering yield and quality (Alisaac & Mahlein, 2023). In Canada alone, at least $1 billion of loss was observed in wheat harvest due to FHB in 2016 (Chin *et al.*, 2023). The most prominent mycotoxin found in wheat due to the FHB is deoxynivalenol (DON). Structurally, DON belongs to the trichothecene family of mycotoxins, characterised by a tetracyclic ring structure with three hydroxyl (-OH) groups (Figure 1). Its chemical name, 12,13-epoxy-3α,7α,15-trihydroxytrichothec-9-en-8-on, reflects its complex molecular structure (Kamle *et al.,* 2022; Nagl & Schatzmayr, 2015). The hydroxyl groups are integral to DON's toxic effects, facilitating interaction with cellular components and disrupting essential biochemical processes. Deoxynivalenol is also considered heat stable, remaining intact even at high temperatures during food processing (80-120°C) (Kabak, 2009). Other mycotoxins found in wheat due to FHB are Enniatin A and B, T-2, HT-2 and moniliformin. Other mycotoxins associated with FHB in wheat include Enniatin A and B, T-2 toxin, HT-2 toxin, and moniliformin (Janaviciene *et al.,* 2023).

Figure 1 Chemical structure of Deoxynivalenol (DON)

If consumed beyond a certain quantity, DON can pose several acute health problems like diarrhoea, abdominal pain, headache, and fever (Ji *et al.,* 2019). Some studies have also shown that prolonged consumption of DON can reduce body weight and prevent weight gain (Tomaszewska *et al.,* 2016; Wellington *et al.,* 2020). According to the Canadian Food Inspection Agency (CFIA), the maximum permissible limit of DON in unclean soft wheat intended for human consumption is 2 ppm. For poultry, the limit is 5 ppm in the final diet, while for swine, it is 1 ppm. Due to these reasons, the nutritional value of *Fusarium*-infected wheat is often undervalued. However, if these nutrients can be recovered, they could alleviate the economic losses for wheat producers and contribute to food security.

Black soldier fly larvae (*Hermetia illucens*; BSFL) are insect species from the tropical/subtropical western hemisphere and Australia (Oliveira *et al.,* 2015). BSFL is known to feed on diverse organic substrates and convert them into nutrient-rich body mass. The dried BSFL biomass contains about 36% – 43% crude protein and 22% – 40% crude lipids, depending upon the feeding substrate used for BSFL rearing (Spranghers *et al.,* 2017). The nutritional value and practical rearing conditions have placed BSFL as a selected candidate source of nutrients for domesticated animals such as poultry, swine, and fish (Leeper *et al.,* 2022; Newton *et al.,* 1977; Onsongo *et al.,* 2018; Rawski *et al.,* 2020). Moreover, the larvae are saprophagous, which means a wide variety of underutilised organic substrates can be utilised to grow them (Gold *et al.,* 2018; Rehman *et al.,* 2019; Sheppard *et al.,* 2002), potentially reducing the overall cost of feed production. Several studies have shown that BSFL tend to feed on organic substrates contaminated with mycotoxins without exhibiting detrimental effects on its performance. The accumulation of mycotoxins in the larvae body was also found to be minimal and, in some cases, below the detection limit compared to the initial feed concentrations (Bosch *et al.,* 2017; Camenzuli *et al.,* 2018; Gulsunoglu *et al.,* 2019; Meijer *et al.,* 2019). Gulsunoglu *et al.* (2019) analysed the DON concentration in 12-day-old BSFL raised on FDK-based diets and found that the accumulation of DON in the larval biomass was minimal. Camenzuli *et al.* (2018) also analysed the DON concentration in BSFL raised on three distinct

diets artificially spiked by the DON and found minimal DON accumulation in the larvae, similar to the previously mentioned study. One of the major reasons behind the minimal accumulation of mycotoxins is the enzymes present in the BSFL gut. Meijer *et al.* (2019) showed that cytochrome P450s were responsible for the conversion of aflatoxin B1 (AFB1) into a less toxic metabolite, AFP1, whereas a cytoplasmic reductase may have converted the AFB1 into Aflatoxicol, another AFB1 metabolite with lower toxicity. Similar enzymatic activities may have reduced other mycotoxins in the BSFL biomass. Another factor to consider is that BSFL stops feeding as early pupae, and intestinal contents are slowly defecated, lowering the overall content of the contaminated feed, and hence the mycotoxins, in the BSFL gut.

Another important aspect is analysing the behaviour of BSFL in response to feed based on its mycotoxin concentration. Studies have shown that animals like swine tend to have lower feed intake when exposed to feeds contaminated with DON. For instance, Dänicke *et al.* (2017) used Fusarium-damaged kernel (FDK)-based diets (5 ppm DON) and healthy wheat-based diets to feed piglets, finding a significant reduction in feed intake for the FDK-based diets. Similarly, Wellington *et al.* (2020) reported that wheat-barley-soybean meal-based diets with varying DON concentrations (0, 1, 3, and 5 ppm) resulted in reduced feed intake as DON concentration increased in the diets. These findings indicate that animals are sensitive to DON concentrations in their feed. Given this sensitivity, it is crucial to understand the effect of DON concentration on the feeding behaviour of BSFL, especially since they are cultivated for feeding purposes. Although BSFL can grow in a wide range of organic substrates, studies have shown that insects tend to prefer certain feeds over others. Parodi *et al.* (2020) showed that BSFL preferred pig manure over the mass-rearing diet. When discussing the preference based on the mycotoxin concentration, a study conducted by Ochoa Sanabria *et al.* (2019) showed that yellow mealworm larvae did not have any feed preferences based on their DON concentrations. However, no such study has observed the behavioural differences of BSFL towards feeds with different mycotoxin concentrations. The insights gained from this research could help develop innovative feeding strategies suitable for industrial application.

This study aims to evaluate, firstly, whether BSFL prefers *Fusarium*-damaged wheat kernels (FDK) over uninfected grain. Secondly, to evaluate the performance of BSFL fed on different concentrations of DON FDK-diets diets. Lastly, the accumulation of DON in BSFL when fed on the experimental diets.

2 Materials and methods

2.1 Materials

The first instar BSFL were sourced from the BSF colony maintained at Enterra Feed Corporation, Langley, British Columbia, Canada. The FDK was supplied by Dr Rex Newkirk, and the control wheat (non-contaminated) was supplied by Dr Pierre Hucl from the University of Saskatchewan. Wheat germ and wheat bran, manufactured by Rogers Foods (Armstrong, BC), were obtained from a local grocery market. The 96-vial enzyme-linked immunosorbent assay (ELISA) kit for DON analysis was purchased from Romer Labs (Vancouver, BC, Canada) under the product name 'AgraQuant® Deoxynivalenol Plus 0.25/5.0 ELISA kit'. ACS-grade petroleum ether (for crude lipid extraction) and ethanol solution (for surface sanitisation) were obtained from Fisher Scientific (Pittsburgh, PA, USA)

2.2 Feed Preparation

Four feed preparations with DON concentrations of 0 ppm (control; Fc), 4.49 ± 0.08 ppm (F1), 6.04 ± 0.02 ppm (F2), and 6.83 ± 0.04 ppm (F3) were formulated using Allix3, a least-cost formulation software (A-Systems, Versailles, France). The formulation was designed to create four different feeds with increasing concentrations of DON while maintaining comparable macronutrient composition across all feed preparations. This ensured that any differences in BSFL growth parameters could be directly attributed to the varying DON concentrations. The ingredients used for feed formulation were FDK (12.3 ppm naturally contaminated DON), healthy wheat (0 ppm DON), wheat germ, wheat bran, and distilled water. Before mixing, FDK and healthy wheat were soaked overnight in different containers for separate feeds in a specific amount of water to ensure a final water content of 66.7%. Once formulated, the feed was ground using a food processor (Black Decker, Model: FP3300SKT) to facilitate easier consumption by the larvae. The specific proportions of ingredients used in the feed preparations are presented in Table 1, while the final nutrient composition (dry basis) of each preparation is shown in Table 2.

Table 1 Composition of ingredients used for feed formulation.

Feed	Water (%)	Infected wheat (%)	Healthy wheat (%)	Wheat germ (%)	Wheat bran (%)
Fc	66.7	0.0	24.3	5.0	4.0
F1	66.7	7.9	16.4	5.0	4.0
F2	66.7	15.8	8.5	5.0	4.0
F3	66.7	23.7	0.6	5.0	4.0

Fc – Control feed; F1-F3 – FDK-based feeds with increasing DON concentrations, where F1 contains the lowest DON concentration and F3 the highest.

Table 2 Proximate composition (dry basis) of feed formulations.[1]

Feed	Crude Lipid (%)	Crude Protein (%)	Carbohydrates (%)	Ash (%)
Fc	2.0 ± 0.1^{b}	20.9 ± 0.2^{a}	74.6 ± 0.2^{c}	2.5 ± 0.1^{a}
F1	2.0 ± 0.0^{b}	19.1 ± 0.1^{b}	76.1 ± 0.3^{bc}	2.7 ± 0.2^{a}
F2	2.5 ± 0.0^{a}	17.4 ± 0.5^{c}	77.1 ± 0.9^{b}	3.0 ± 0.3^{a}
F3	2.6 ± 0.0^{a}	15.2 ± 0.0^{d}	79.3 ± 0.3^{a}	3.0 ± 0.3^{a}

[1] The data is shown as mean ± standard deviation (n = 3). Means for each nutrient marked with different lowercase alphabets are significantly different from each other ($p<0.05$). Fc – Control feed; F1-F3 – FDK-based feeds with increasing DON concentrations, where F1 contains the lowest DON concentration and F3 the highest.

2.3 Preference trial

This trial aimed to ascertain the preference of BSFL towards a specific feed based on its DON concentration. For this study, the 3rd instar BSFL, approximately 14 – 16 mm long, were chosen and placed in three different setups.

In the first setup, all four feed preparations were filled in separate aluminium dishes (5.7 mm diameter, 50 ml capacity), and 10 BSFL were introduced into each dish. They were then left undisturbed inside the incubator at 30°C for 60 minutes (Figure 2, part A). This setup aimed to determine whether BSFL exhibited a preference or rejection towards a specific feed. A feed from which most BSFL attempted to escape was presumed to be the least preferable. In the second setup

(Figure 2, part B), 10 BSFL were selected and surrounded by all four feed preparations. After being introduced, they were left undisturbed inside the incubator at 30℃ for 60 minutes, and the final location of the larvae was noted. The positions of the feed preparations were randomised and changed after every run. The third setup mirrored the second one with a minor alteration: fasting larvae. In this setup, BSFL were starved for 24 hours before conducting the preference trial to observe whether starvation affected their behaviour towards the feed. This setup was also conducted inside the incubator at 30℃ for 60 minutes. The purpose of using the second and third setups was to observe the preference of BSFL towards a specific feed when all feed options were available to them.

Each setup was repeated 15 times. After the data collection, the mean number of larvae detected in each feed was calculated. A one-way analysis of variance (ANOVA) was then applied to evaluate any statistical disparities among the groups. To determine the differences in treatment means, Tukey's t-test was conducted, with a significance level set at $p < 0.05$.

Figure 2. Experimental setup used for preference trail.

2.4 BSFL feeding

One hundred healthy BSFL (first instar, about 3 – 5 mm in length), counted with a pair of forceps, were introduced in 75 g of each feed replicate, enclosed in a glass jar with a meshed lid. For each type of feed, 12 biological replicates were prepared. All the jars were stored in the incubator at 30℃, and distilled water was added to each jar every day, ensuring that weight loss due to evaporation was constant. On Days 5, 10, and 15, three replicates were analysed for each type of feed preparation. The BSFLs were removed from the spent feed with forceps, cleaned with distilled water, and dried with paper towels, and their live body weight was recorded. After growth analysis, the BSFL were euthanised by storing them at -20℃. All the samples collected were also stored at -20℃ until further analysis.

2.5 Growth Analysis

The parameters measured to analyse the overall growth of the larvae were live body weight and larvae dimensions. For live body weight analysis, 50 random live larvae were selected from each replicate, and their mean weight was measured, ensuring that the live weight included the population's average weight for each type of feed. For measurement of larvae dimensions, the length and width of larvae were recorded for each replicate via vernier callipers.

2.6 Proximate analysis

The proximate composition of the samples was measured using the AACC methods of analysis. The moisture content of the samples was analysed as per AACC International Method 44-15.02 with slight modifications. The samples were dried overnight at 68℃ in the hot air oven, and the difference in sample weight was used to calculate the moisture content. For crude lipid analysis, AACC international method 30-20.01 was adopted, where Goldfisch extraction was followed, and petroleum ether was used as the extraction solvent. The dried samples were weighed, 0.5 g for BSFL biomass and 5.0 g for feed and spent feed, onto Whatman filter paper (type 1), and the extraction time was 4 hours. The crude protein combustion method, as per AACC International Method 46-30.01, was employed to measure the crude protein content of the samples. The conversion factor of 6.25 was employed to measure the protein content of BSFL biomass, whereas the conversion factor of 5.70 was used for the feed and spent feed (Gulsunoglu *et al.*, 2019). For ash analysis, AACC Approved Method 08-01.01 was adopted, where 3.0 g of dried sample was

placed onto ceramic crucibles and charred onto a hot plate inside the fume hood to remove the volatile organic compounds. These organic compounds can produce much smoke if placed directly inside the muffle furnace. Finally, the charred samples were placed into the muffle furnace at 550℃ overnight. The total carbohydrates (dry basis) were calculated by subtracting the crude lipid, crude protein, and ash content from the total sample weight (dried).

2.7 DON analysis

The DON content of the samples was analysed via enzyme-linked immunosorbent assay (ELISA) kit. All the reagents used for the ELISA came with the kit and were used as instructed by the manual. The samples were also prepared per the kit instructions but with slight modifications. Ground sample (0.5 g) was mixed with 2.5 ml of distilled water (1:5 dilution factor). The dilution factor of 5 was chosen to ensure that the final concentration of samples lies between the upper and lower limit of DON detection for the kit. The samples were centrifuged at 3500 rpm for 3 minutes (Microfuge 20, Beckman Coulter, Brea, CA, United States), and 100 µL of supernatant was added to 200 µl of the conjugate solution in the antibody wells of the micro-ELISA plate provided with the kit. The plate was incubated at room temperature for 10 minutes, and each well was washed 5 times with the wash buffer. Then, 100 µl of the substrate solution was added to each well and incubated at room temperature. After 5 min, 100 µl of the stop solution was added to each well. The absorbance of each well was determined at a wavelength of 450 nm using a Varioskan LUX multimode microplate reader, which incorporated a differential filter set at 630 nm for reference. This differential filter at 630 nm assisted in minimising background signals or interference, ensuring more accurate absorbance measurements in the experiment. The control samples provided with the kit were treated using the same method to provide the reference curve to determine the DON concentrations of the samples.

2.8 Statistical analysis

Each feeding setup consisted of 3 biological replicates, with 2 technical duplicates for each biological replicate. This approach provided a total of six replicates for each category (n=6), ensuring accuracy and precision. For the analysis of the nutrient composition of the initial feed preparation, three technical replicates were used (n=3). The results were presented as a mean with the standard deviation, and the statistical significance was analysed using the one-way analysis of variance (ANOVA). The statistical difference in the treatment means was determined via Tukey's t-test at $p < 0.05$. The software employed for the statistical analysis was OriginLab's *Origin Pro 2023* (Northampton, Massachusetts, USA).

3 Results and discussion

3.1 Preference trial

The outcome of the preference trials did not observe any predilection of BSFL towards any feed preparation (Table 3). For the first setup, none of the BSFL tried to escape from any treatment except for a few cases, but no pattern was observed. In the second and third setups, the final position of BSFL were different after every run and were not attracted to any particular feeds, suggesting that the difference in DON concentration of the feed did not affect the preference of BSFL towards any feed. Parodi *et al.* (2020) showed that BSFL preferred pig manure over a mass-rearing diet. It shows that the BSFL may prefer a feed based on its smell, palatability, moisture content, and particle size. Since no study is available to show that DON contributes to any aroma or taste, all the feed preparations may have been similar from the perspective of BSFL and hence,

no preference was given to any feed. However, since the BSFL did not try to escape from the feed, it was assumed that all feed preparations were acceptable to BSFL. Similar results were reported by Ochoa-Sanabria *et al.* (2019), where four FDK-based diets with different DON concentrations were utilised to feed the yellow mealworm larvae, and the larvae showed no feeding preference. These results suggest that insects, such as BSFL and yellow mealworm larvae, may not possess sensory organs as advanced as those of animals, like swine, to exhibit sensitivity to feed based on their DON concentration (Dänicke *et al.*, 2017; Wellington *et al.*, 2020).

Table 3 Number of larvae found in each feed during the preference trial.

Runs	**Setup 1**				**Setup 2**					**Setup 3**				
	Fc	**F1**	**F2**	**F3**	**Fc**	**F1**	**F2**	**F3**	**N[1]**	**Fc**	**F1**	**F2**	**F3**	**N[1]**
1	10	9	10	8	11	8	4	6	1	10	6	4	9	1
2	7	9	9	10	6	3	6	14	1	4	9	12	3	2
3	9	9	10	8	7	7	9	7	0	6	5	14	5	0
4	10	10	10	10	7	3	10	9	1	3	11	7	8	1
5	9	9	10	10	4	12	5	7	2	7	4	12	7	0
6	8	10	9	10	3	6	11	8	2	2	3	8	16	1
7	6	10	9	8	9	8	6	5	2	5	12	6	7	0
8	10	9	9	8	8	8	5	6	3	11	6	10	3	0
9	9	10	9	10	4	8	12	6	0	1	9	14	6	0
10	8	9	9	9	5	14	4	7	0	9	11	8	2	0
11	10	8	9	8	4	13	1	9	3	6	12	7	4	1
12	9	5	10	9	13	5	3	8	1	8	5	8	9	0
13	10	10	9	10	4	9	6	11	0	7	13	3	6	1
14	9	8	10	6	16	3	5	2	4	2	8	4	13	3
15	10	9	10	8	4	4	13	9	0	10	12	5	3	0
Mean[2]	8.9 ± 1.2[a]	8.9 ± 1.3[a]	9.5 ± 0.5[a]	8.8 ± 1.2[a]	7.0 ± 3.8[b]	7.4 ± 3.6[b]	6.7 ± 3.5[b]	7.6 ± 2.7[b]	1.3 ± 1.3[c]	6.1 ± 3.2[d]	8.4 ± 3.4[d]	8.1 ± 3.6[d]	6.7 ± 3.9[d]	0.7 ± 0.9[e]

[1] N: number of larvae found in locations other than the feed.

[2] The data in the last row is shown as mean ± standard deviation (n = 15). Means for each experiment marked with different lowercase alphabets are significantly different from each other (p<0.05). Fc – Control feed; F1-F3 – FDK-based feeds with increasing DON concentrations, where F1 contains the lowest *DON* concentration and F3 the highest.

3.2 DON concentration of the BSFL and spent feed

The DON concentration analysis of BSFL showed that DON remained at levels less than 2 ppm (Table 4). On Day 10, the DON concentration peaked in the biomass, then it reduced to less than 1 ppm on Day 15, below the maximum permissible limit of DON in human foods as per the FDA. Specifically, the DON concentration for BSFL fed with F1, F2 and F3 were 0.61 ± 0.06 ppm, 0.64 ± 0.16 ppm and 0.87 ± 0.10 ppm, respectively, at Day 15. This trend in DON accumulation can be directly correlated to the feeding behaviour of the BSFL. Until the prepupal stage, the rate of feed

consumption by BSFL was at its maximum, meaning that the inflow of DON was high. The DON concentration in the biomass was also high during this period. After Day 10, the larvae grew entirely and stopped feeding, preparing for pupal metamorphosis. At this stage, the BSFL shed the feed remnants from the gut, which aids in the reduction of DON concentration.

Additionally, this reduction may be associated with the metabolism of DON by BSFL gut and microbiota. There is limited literature regarding DON metabolism in the BSFL gut. Lee & Campbell (2000) studied the in-vitro metabolism of Aflatoxin B1 (AFB1) by navel orangeworm (*Amyelois transitella*) larvae. Their findings suggested that cytochrome P450-dependent monooxygenase and NADPH-dependent cytoplasmic reductase were responsible for the conversion of AFB1 into its metabolites. A similar pathway may be present in the BSFL to convert the DON into less toxic metabolites like 3-acetyl-DON (3-ADON), 15-acetyl-DON (15-ADON) and DON-3-glucoside (D3G). Analysis of these metabolites along with DON can put further light on the metabolism of DON by BSFL.

In the case of the spent feed, the DON concentration in the FDK-based feed remained high or slightly increased at the end of the feeding cycle. For F1, F2, and F3, the initial DON concentration was 4.49 ± 0.11 ppm, 6.04 ± 0.13 ppm, and 6.83 ± 0.06 ppm, respectively. On Day 15, the concentration was 6.41 ± 0.36 ppm, 6.78 ± 0.22 ppm, and 6.50 ± 0.14 ppm for the respective spent feeds. A study by Berthiller *et al.* (2011) showed that the intestinal bacteria belonging to the genera *Lactobacillus, Enterococcus, Enterobacter,* and *Bifidobacterium* were responsible for converting D3G into DON. It may be possible that the D3G present in the FDK was converted into DON while passing through the BSFL gut, increasing the overall concentration of DON in the spent feed. Another possibility could be the presence of *Fusarium spp.* in the FDK. While feeding, the environmental temperature was set at 30 °C for 15 days, which was good for promoting microbial growth and may also have increased the *Fusarium spp.* colonies already present in the feed. Since *Fusarium spp.* is responsible for the production of DON in the FDK, they may have continued to produce more DON given the favourable growth conditions.

Table 4. DON concentrations in feed, spent feed at Day 15, and in the BSFL biomass for Day 5, 10, and 15. [1]

Feed	Feed (ppm)	Spent feed (ppm)	Biomass (ppm)		
	Day 0	Day 15	Day 5	Day 10	Day 15
Fc	LQ	LQ	LQ	LQ	LQ
F1	4.49 ± 0.11^{d}	6.41 ± 0.36^{bc}	0.5 ± 0.01^{hi}	0.82 ± 0.14^{gh}	0.61 ± 0.06^{h}
F2	6.04 ± 0.13^{c}	6.78 ± 0.22^{a}	1.04 ± 0.01^{fgh}	1.03 ± 0.24^{g}	0.64 ± 0.16^{h}
F3	6.83 ± 0.06^{ab}	6.5 ± 0.14^{abc}	1.55 ± 0.04^{ef}	2 ± 0.11^{e}	0.87 ± 0.10^{gh}

[1] The data is shown as mean ± standard deviation (n = 6). Means for each experiment marked with different lowercase alphabets are significantly different from each other ($p<0.05$). LQ means below the limit of quantification. Fc – Control feed; F1-F3 – FDK-based feeds with increasing DON concentrations, where F1 contains the lowest DON concentration and F3 the highest.

3.3 Growth analysis of the BSFL

In examining the impact of DON concentration on BSFL live body mass, notable differences emerged between the groups fed on a diet containing FDK and the control (Table 5). The larvae raised on FDK-based feed showed superior growth, as evidenced by significantly higher average weights for F1, F2, and F3 on Days 5, 10, and 15 compared to their counterparts on the control

diet (Fc) (Table 4). However, by Day 15, the larvae weight difference between control and FDK-based feeds becomes narrower. It is important to note that smaller differences at Day 15 do not necessarily imply comparable growth rates among all BSFL grown on different diets. Around Day 15, fully-grown BSFL metamorphose into prepupae, leading to a slight drop in overall larval body weight. It happens due to the non-feeding behaviour of BSFL after the initiation of the prepupal stage (Georgescu *et al.,* 2020). The accelerated growth observed in BSFL consuming an FDK-based diet suggests an earlier progression to the prepupal stage. Consequently, reduced weight gain at this point appears comparable to BSFL raised on the control diet (Fc), potentially still in the later phase of the larval stage. This hypothesis gains support from the observed shift in the colouration of BSFL at different life cycle stages. At the pre-pupating stage, BSFL darkens their body colour, eventually attaining a dark brown hue. The prevalence of darker BSFL at days 10 and 15 was notably higher in the FDK-based diet group than in the control group (Fc), indicating a more rapid growth rate in BSFL exposed to FDK-based diets. The reason behind the faster growth rate of BSFL in the case of FDK-based diets is likely associated with the presence of *Fusarium spp.* in the feed. Studies have shown that Fusarium species express digestive enzymes that enhance the digestibility of proteins and carbohydrates in their substrate (Bacala *et al.,* 2021; Dänicke *et al.,* 2003). A wide array of hydrolytic enzymes produced by *F. graminearum*, including cell wall-degrading enzymes, are involved in plant cell wall penetration, which may also contribute to improved overall nutrient digestibility (Moonjely *et al.,* 2023; Zhao *et al.,* 2013). It is also established that the BSFL growth parameters are heavily dependent on the nutrient digestibility of the feed substrate. Kuttiyatveetil *et al.* (2019) have shown that the BSFL feeding on fermented substrate had a faster growth rate as compared to the unfermented feed, where the dry weight of BSFL, on Day 12, raised on unfermented flaxseed was around 66 mg, whereas it reached up to 83 mg for flaxseed fermented with *L. plantarum* and *A. oryzae*. These factors may have promoted the growth rate of BSFL feeding on the FDK-based diets compared to the control diet.

Table 5 Live body weight and dry weight of BSFL recorded on the Day 5, 10 and 15 of feeding for different diet preparations. [1]

Feed	Day 5	Day 10	Day 15
	Live body weight (mg) (Dry weight) (mg)	**Live body weight (mg) (Dry weight) (mg)**	**Live body weight (mg) (Dry weight) (mg)**
Fc	53.3 ± 2.7^{h} (14.1 ± 0.7^{g})	137.1 ± 5.2^{e} (36.4 ± 1.4^{e})	177.6 ± 4.2^{ab} (50.5 ± 1.2^{b})
F1	66.8 ± 4.6^{fg} (17.9 ± 1.2^{f})	166.3 ± 6.2^{c} (44.3 ± 1.6^{c})	180 ± 1.9^{a} (51.4 ± 0.5^{ab})
F2	62.8 ± 1.6^{g} (17.2 ± 0.5^{f})	151.7 ± 4.3^{d} (39.7 ± 1.1^{d})	183.3 ± 5.6^{a} (52.6 ± 1.6^{ab})
F3	71.5 ± 4.3^{f} (18.7 ± 1.1^{f})	169.1 ± 5.7^{bc} (45.1 ± 1.5^{c})	185 ± 3.2^{a} (53.6 ± 0.9^{a})

[1] The data is shown as mean ± standard deviation (n = 6). Means for each experiment marked with different lowercase alphabets are significantly different from each other ($p<0.05$). The alphabets that show statistical differences among live body weights are not comparable to those used for dry weight values in brackets. Fc – Control feed; F1-F3 – FDK-based feeds with increasing DON concentrations, where F1 contains the lowest DON concentration and F3 the highest.

There were no significant differences in larvae length and width across the treatments ($p<0.05$). Since the dimensions of BSFL do not change much after a specific point of growth, the difference in length and width of larvae across the feeds was expected to be negligible. Larvae length (and width) on Day 5, 10 and 15 were 14.2 ± 0.9 mm (3.6 ± 0.5 mm), 17.7 ± 0.4 mm (4.7 ± 0.6 mm), 19.6 ± 0.5 mm (5.1 ± 0.2 mm), respectively.

3.4 Proximate composition of the BSFL and spent feed

The accumulation of protein and lipids in the BSFL biomass at different feeding stages elucidates the larvae's growth pattern (Figure 3). In the case of control feed, the crude protein content was 60.5% ± 0.0%, 46.9% ± 1.6% and 39.2% ± 0.8% at Day 0, 5 and 10, respectively, whereas the crude lipid content was 18.0% ± 0.4%, 21.3% ± 1.0% and 39.1% ± 1.6% for the respective days. The same pattern was observed in F1, F2, and F3, where the crude protein proportion decreased, and the crude lipid proportion increased with time in the BSFL biomass. However, the overall amount of both nutrients increased over time (Figure 3). The shift in proportions can be attributed to the feeding behaviour of BSFL. Initially, feeding and body development progress simultaneously, allowing the larvae to meet their energy requirements through continuous feeding while storing protein for growth and development. However, during the prepupal stage, BSFL ceases feeding. Consequently, storing energy for the latter part of the life cycle becomes crucial before the larvae's development into prepupae. Given that lipids are the most efficient in storing energy, this shift in proportions over time may be a result of an increased emphasis on lipid accumulation. A similar trend was observed by Gulsunoglu *et al.* (2019), where the protein concentration for the control feed dropped from 56.2% on Day 0 to 37.1% on Day 5, and the lipid proportion increased from 17.9% to 41.1% for respective days.

The carbohydrate content in the BSFL biomass also increased over time, where the concentration at Day 5 was 2.1 ± 0.0 mg/larva and reached up to 9.1 ± 0.9 mg/larva at Day 15 for the control feed. A similar trend was observed in F1, F2, and F3, where the final concentration, at Day 15, reached 7.9 ± 1.2 mg/larva, 8.4 ± 1.0 mg/larva, and 7.9 ± 0.6 mg/larva, respectively.

The ash content followed a similar trend where the initial content for Fc, F1, F2, and F3 was 0.9 ± 0.0 mg/larva, 1.1 ± 0.1 mg/larva, 1.0 ± 0.0 mg/larva and 1.0 ± 0.0 mg/larva respectively and reached up to 1.5 ± 0.1, 1.7 ± 0.2, 1.6 ± 0.2 and 1.6 ± 0.2 mg/larva, respectively.

Figure 3. Proximate composition (mg per larva) of BSFL in dry weight raised on Fc, F1, F2, and F3 at Day 5, 10, and 15.

4 Conclusion and future studies

BSFL demonstrated the ability to thrive on substrates with varying concentrations of DON without experiencing any adverse effects on its growth parameters. The accumulation of DON in the biomass was insignificant. Regarding nutritional quality, no significant differences were observed in the BSFL biomass based on the DON concentration in the feed. These indicate that BSFL can recover nutrients from DON-contaminated substrates without the DON adversely affecting its performance. The preference trial supported the conclusion that DON-contaminated substrates can be effectively utilised on a larger scale without BSFL exhibiting any rejection towards the feed. Thus, BSFL can be a promising tool for recovering nutrients from underutilised wheat production streams.

Future studies are crucial to study the metabolism of DON within the BSFL and investigate the toxicity of its metabolites. Additionally, it is important to determine any traceable concentrations of mycotoxins in livestock that consume BSFL raised on Fusarium-infected feeds. These investigations will provide insight into the thresholds at which DON-contaminated substrates can be utilised as a feed for BSFL and, consequently, as an ingredient for animal feed.

In the context of using FDK as a substrate for BSFL cultivation, future studies should investigate the potential carryover of *Fusarium* spp. and other microorganisms into the BSFL biomass. Understanding how these microbes interact with and persist in the insect biomass will be crucial for ensuring the microbial safety of BSFL as a feed ingredient. Furthermore, a detailed examination of the metabolism of DON and other mycotoxins in the BSFL gut is necessary to evaluate how these toxins are processed or reduced by the larvae. Additionally, it is important to assess the impact of feeding animals with BSFL grown on FDK on their health, morphology, and nutritional profile. This includes evaluating any potential carryover of mycotoxins from the BSFL to animal products and determining the overall nutritional benefits of using these larvae as feed. Scaling up the use of FDK for industrial BSFL cultivation also requires an assessment of economic feasibility. This includes analyses of the costs associated with procuring and processing FDK, as well as the potential revenue from BSFL and their by-products.

5 Acknowledgements

This study was partly supported by the Alberta Agriculture Funding Consortium grant. The authors appreciate Drs. Randy Kutcher, Lipu Wang, and Rex Newkirk of the University of Saskatchewan for their assistance in DON analysis.

6 References

Alisaac, E., & Mahlein, A.-K. (2023). Fusarium Head Blight on Wheat: Biology, Modern Detection and Diagnosis and Integrated Disease Management. *Toxins*, *15*(3), 192. https://doi.org/10.3390/toxins15030192

Bacala, R., Fu, B. X., Cordova, K., & Hatcher, D. W. (2021). Wheat Fusarium Protease Specificity and Effect on Dough Properties. *Foods*, *10*(7), 1585. https://doi.org/10.3390/foods10071585

Berthiller, F., Krska, R., Domig, K. J., Kneifel, W., Juge, N., Schuhmacher, R., & Adam, G. (2011). Hydrolytic fate of deoxynivalenol-3-glucoside during digestion. *Toxicology Letters*, *206*(3), 264–267. https://doi.org/10.1016/j.toxlet.2011.08.006

Bosch, G., Van Der Fels-Klerx, H. J., De Rijk, T. C., & Oonincx, D. G. A. B. (2017). Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (*Hermetia illucens*) and yellow mealworms (*Tenebrio molitor*). *Toxins*, *9*(6). https://doi.org/10.3390/toxins9060185

Camenzuli, L., Van Dam, R., de Rijk, T., Andriessen, R., Van Schelt, J., & Van der Fels-Klerx, H. (2018). Tolerance and Excretion of the Mycotoxins Aflatoxin B1, Zearalenone, Deoxynivalenol, and Ochratoxin A by *Alphitobius diaperinus* and *Hermetia illucens* from Contaminated Substrates. *Toxins*, *10*(2), 91. https://doi.org/10.3390/toxins10020091

Chin, T., Pleskach, K., Tittlemier, S. A., Henriquez, M. A., Bamforth, J., Withana Gamage, N., Ashfaq, T., Lee, S.-J., Kurera, M. S., Patel, B., & Walkowiak, S. (2023). A status update on fusarium head blight on Western Canadian wheat. *Canadian Journal of Plant Pathology*, *45*(3), 277–289. https://doi.org/10.1080/07060661.2023.2177352

Dänicke, S., Beineke, A., Berk, A., & Kersten, S. (2017). Deoxynivalenol (DON) Contamination of Feed and Grinding Fineness: Are There Interactive Implications on Stomach Integrity and Health of Piglets? *Toxins*, *9*(1), 16. https://doi.org/10.3390/toxins9010016

Dänicke, S., Matthes, S., Halle, I., Ueberschär, K. H., Döll, S., & Valenta, H. (2003). Effects of graded levels of Fusarium toxin-contaminated wheat and of a detoxifying agent in broiler diets on performance, nutrient digestibility and blood chemical parameters. *British Poultry Science*, *44*(1), 113–126. https://doi.org/10.1080/0007166031000085300

Georgescu, B., Struti, D., Papuc, T., Ladosi, D., & Boaru, A. (2020). Body weight loss of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) during development in non-feeding stages: Implications for egg clutch parameters. *European Journal of Entomology*, *117*, 216–225. https://doi.org/10.14411/eje.2020.023

Gold, M., Tomberlin, J. K., Diener, S., Zurbrügg, C., & Mathys, A. (2018). Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: A review. In *Waste Management* (Vol. 82, pp. 302–318). Elsevier Ltd. https://doi.org/10.1016/j.wasman.2018.10.022

Gulsunoglu, Z., Aravind, S., Bai, Y., Wang, L., Kutcher, H. R., & Tanaka, T. (2019). Deoxynivalenol (DON) Accumulation and Nutrient Recovery in Black Soldier Fly Larvae (*Hermetia illucens*) Fed Wheat Infected with *Fusarium* spp. *Fermentation*, *5*(3), 83. https://doi.org/10.3390/fermentation5030083

Janaviciene, S., Venslovas, E., Kadziene, G., Matelioniene, N., Berzina, Z., Bartkevics, V., & Suproniene, S. (2023). Diversity of Mycotoxins Produced by *Fusarium* Strains Infecting Weeds. *Toxins*, *15*(7), 420. https://doi.org/10.3390/toxins15070420

Ji, F., He, D., Olaniran, A. O., Mokoena, M. P., Xu, J., & Shi, J. (2019). Occurrence, toxicity, production and detection of Fusarium mycotoxin: a review. *Food Production, Processing and Nutrition*, *1*(1), 6. https://doi.org/10.1186/s43014-019-0007-2

Kabak, B. (2009). The fate of mycotoxins during thermal food processing. *Journal of the Science of Food and Agriculture*, *89*(4), 549–554. https://doi.org/10.1002/jsfa.3491

Kamle, M., Mahato, D. K., Gupta, A., Pandhi, S., Sharma, B., Dhawan, K., Vasundhara, Mishra, S., Kumar, M., Tripathi, A. D., Rasane, P., Selvakumar, R., Kumar, A., Gamlath, S., & Kumar, P. (2022). Deoxynivalenol: An Overview on Occurrence, Chemistry, Biosynthesis, Health Effects and Its Detection, Management, and Control Strategies in Food and Feed. *Microbiology Research*, *13*(2), 292–314. https://doi.org/10.3390/microbiolres13020023

Kuttiyatveetil, J. R. A., Mitra, P., Goldin, D., Nickerson, M. T., & Tanaka, T. (2019). Recovery of residual nutrients from agri-food byproducts using a combination of solid-state fermentation and insect rearing. *International Journal of Food Science & Technology*, *54*(4), 1130–1140. https://doi.org/10.1111/ijfs.14015

Lee, S.-E., & Campbell, B. C. (2000). In vitro metabolism of aflatoxin B1 by larvae of navel orangeworm, *Amyelois transitella* (Walker) (Insecta, Lepidoptera, Pyralidae) and codling moth, *Cydia pomonella* (L.) (Insecta, Lepidoptera, Tortricidae). *Archives of Insect Biochemistry and Physiology*, *45*(4), 166–174. https://doi.org/10.1002/1520-6327(200012)45:4<166::AID-ARCH4>3.0.CO;2-8

Leeper, A., Benhaïm, D., Smárason, B., Knobloch, S., Ómarsson, K. L., Bonnafoux, T., Pipan, M., Koppe, W., Björnsdóttir, R., & Øverland, M. (2022). Feeding black soldier fly larvae (Hermetia illucens) reared on organic rest streams alters gut characteristics of Atlantic salmon (Salmo salar). *Journal of Insects as Food and Feed*, *8*(11), 1355–1372. https://doi.org/10.3920/JIFF2021.0105

Meijer, N., Stoopen, G., van der Fels-Klerx, H. J., van Loon, J. J. A., Carney, J., & Bosch, G. (2019). Aflatoxin B1 Conversion by Black Soldier Fly (*Hermetia illucens*) Larval Enzyme Extracts. *Toxins*, *11*(9), 532. https://doi.org/10.3390/toxins11090532

Mielniczuk, E., & Skwaryło-Bednarz, B. (2020). Fusarium head blight, mycotoxins and strategies for their reduction. *Agronomy*, *10*(4), 11–15. https://doi.org/10.3390/agronomy10040509

Moonjely, S., Ebert, M., Paton-Glassbrook, D., Noel, Z. A., Roze, L., Shay, R., Watkins, T., & Trail, F. (2023). Update on the state of research to manage Fusarium head blight. In *Fungal Genetics and Biology* (Vol. 169). Academic Press Inc. https://doi.org/10.1016/j.fgb.2023.103829

Nagl, V., & Schatzmayr, G. (2015). Deoxynivalenol and its masked forms in food and feed. *Current Opinion in Food Science*, *5*, 43–49. https://doi.org/10.1016/j.cofs.2015.08.001

Newton, G. L., Booram, C. V., Barker, R. W., & Hale, O. M. (1977). Dried *Hermetia Illucens* Larvae Meal as a Supplement for Swine. *Journal of Animal Science*, *44*(3), 395–400. https://doi.org/10.2527/jas1977.443395x

Oliveira, F., Doelle, K., List, R., & Reilly, J. R. O. (2015). Assessment of Diptera : Stratiomyidae , genus *Hermetia illucens* (L., 1758) using electron microscopy. *Journal of Entomology and Zoology Studies*, *3*(5), 147–152. https://www.researchgate.net/publication/305378530

Onsongo, V. O., Osuga, I. M., Gachuiri, C. K., Wachira, A. M., Miano, D. M., Tanga, C. M., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. K. M. (2018). Insects for Income Generation Through Animal Feed: Effect of Dietary Replacement of Soybean and Fish Meal With Black Soldier Fly Meal on Broiler Growth and Economic Performance. *Journal of Economic Entomology*, *111*(4), 1966–1973. https://doi.org/10.1093/jee/toy118

Rawski, M., Mazurkiewicz, J., Kierończyk, B., & Józefiak, D. (2020). Black Soldier Fly Full-Fat Larvae Meal as an Alternative to Fish Meal and Fish Oil in Siberian Sturgeon Nutrition: The Effects on Physical Properties of the Feed, Animal Growth Performance, and Feed Acceptance and Utilization. *Animals*, *10*(11), 2119. https://doi.org/10.3390/ani10112119

Rehman, K. ur, Ur Rehman, R., Somroo, A. A., Cai, M., Zheng, L., Xiao, X., Ur Rehman, A., Rehman, A., Tomberlin, J. K., Yu, Z., & Zhang, J. (2019). Enhanced bioconversion of dairy and chicken manure by the interaction of exogenous bacteria and black soldier fly larvae. *Journal of Environmental Management*, *237*, 75–83. https://doi.org/10.1016/j.jenvman.2019.02.048

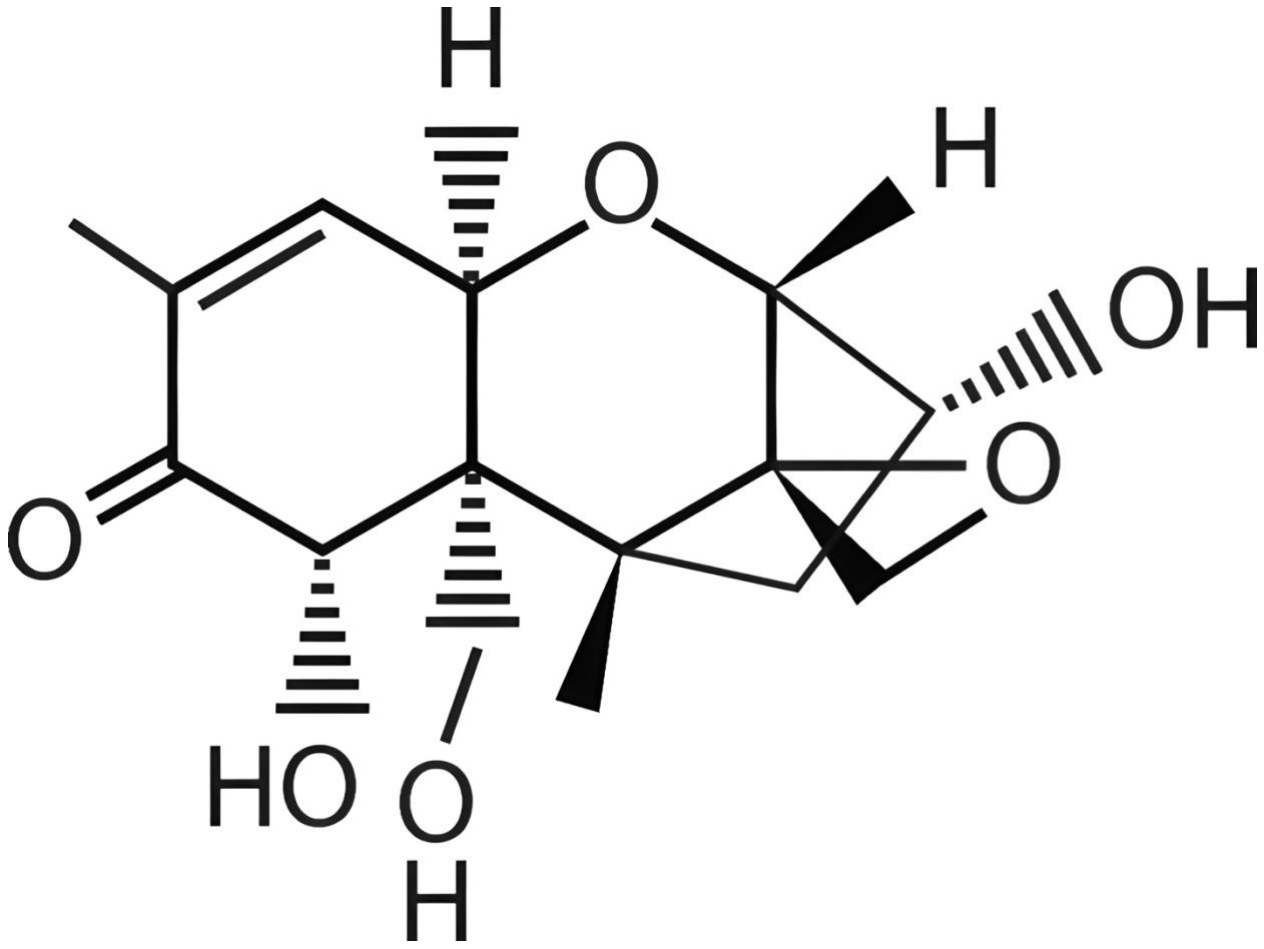
Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., & Sumner, S. M. (2002). Rearing Methods for the Black Soldier Fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, *39*(4), 695–698. https://doi.org/10.1603/0022-2585-39.4.695

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P., & De Smet, S. (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*, *97*(8), 2594–2600. https://doi.org/10.1002/jsfa.8081

Tomaszewska, E., Muszyński, S., Dobrowolski, P., Kostro, K., Taszkun, I., Żmuda, A., Blicharski, T., & Kędzia, P. (2016). Bentonite diminishes DON-induced changes in bone development in mink dams. *Journal of Veterinary Research*, *60*(3), 349–355. https://doi.org/10.1515/jvetres-2016-0033

Wellington, M. O., Bosompem, M. A., Petracek, R., Nagl, V., & Columbus, D. A. (2020). Effect of long-term feeding of graded levels of deoxynivalenol (DON) on growth performance, nutrient utilization, and organ health in finishing pigs and DON content in biological samples. *Journal of Animal Science*, *98*(12). https://doi.org/10.1093/jas/skaa378

Zhao, Z., Liu, H., Wang, C., & Xu, J. R. (2013). Comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi. *BMC Genomics*, *14*(1). https://doi.org/10.1186/1471-2164-14-274



507 Fig 1.

508

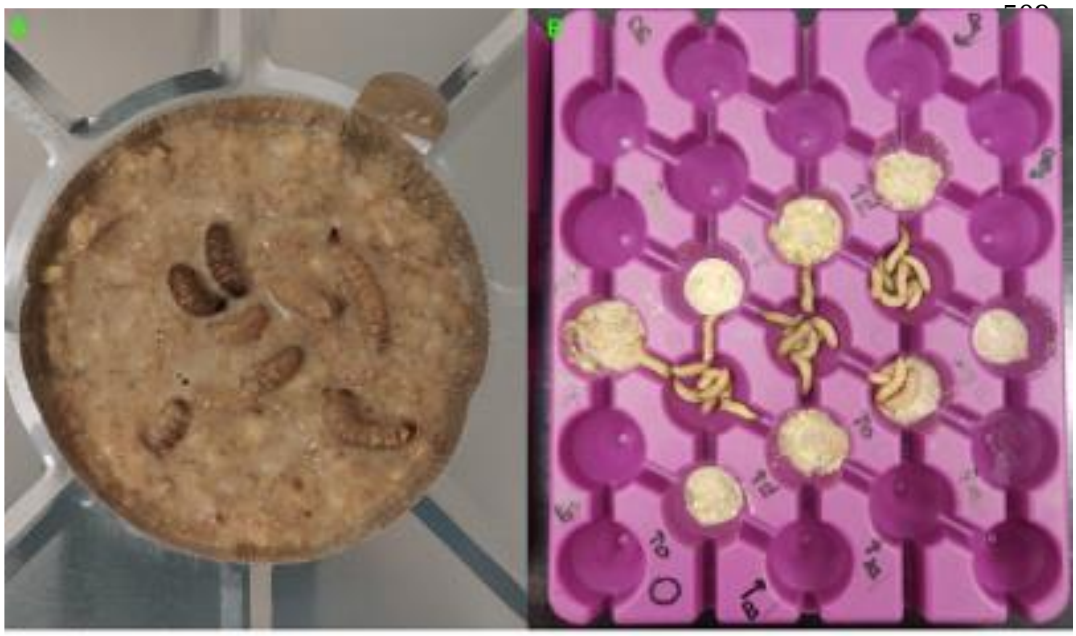


Figure 2

Note: The first image (A) is the design used for the first setup, whereas the second image (B) is the design used for the second setup. In setup A, all the feed preparations were filled in separate aluminium pans as shown in the image, and 10 BSFL were introduced in all the pans, whereas in setup B, ten BSFL were introduced in three depressions, adjoinin to all four feed preparations, and the movement of the larvae through the grooves towards the feed preparations was monitored.

517

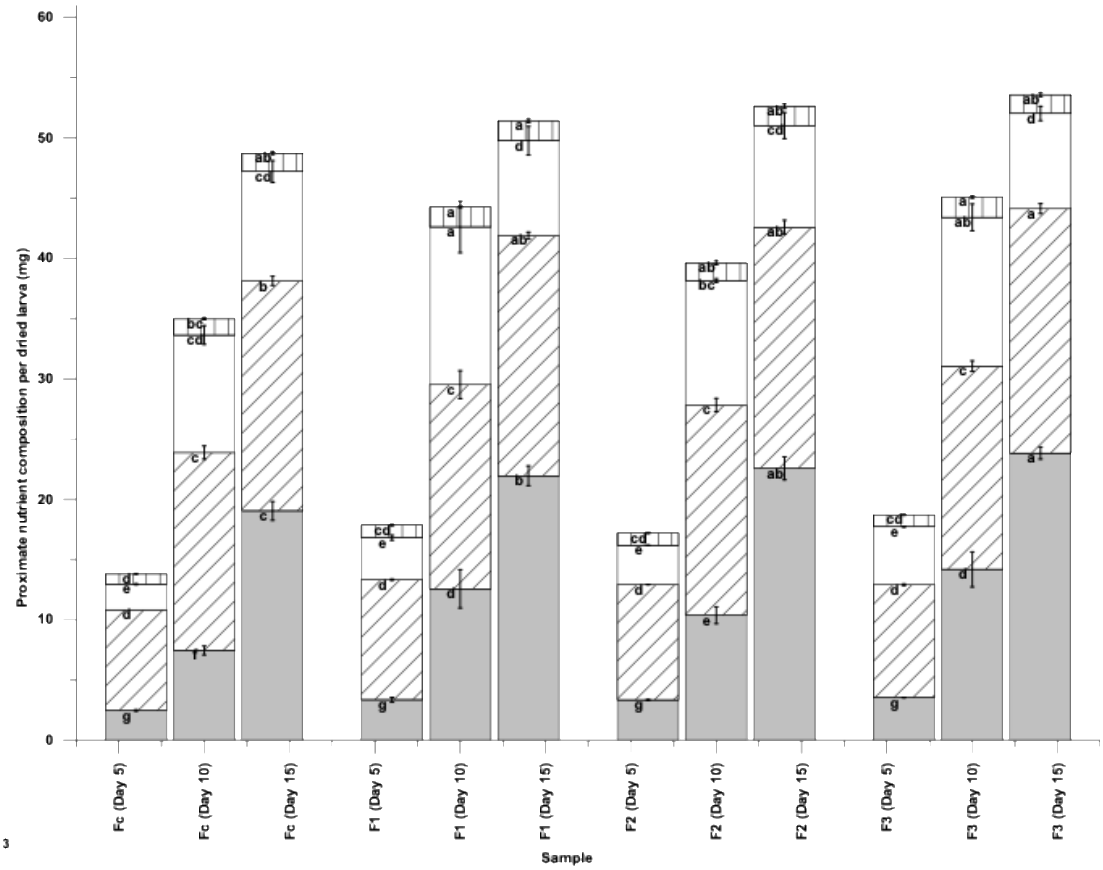


Figure 3

NOTE:

The data is shown as mean ± standard deviation (n = 6), where the standard deviation is depicted as vertical lines at the top of each bar. Means for each value marked with different lowercase alphabets within the same nutrient category are significantly different from each other (p<0 05). The lowercase alphabets are not comparable across different nutrient categories. Each bar is divided into four sections showing four major nutrient categories: vertical hatch (top) – total ash; white – total carbohydrates; angled hatch – total crude protein; grey – total crude lipids.