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Growth performance and nutrient composition of black soldier fly larvae reared on solid-state fermentation substrates with various white rot fungi

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Abstract. *Fitriana EL, Jayanegara A, Astuti DA, Laconi EB. 2022. Growth performance and nutrient composition of black soldier fly larvae reared on solid-state fermentation substrates with various white rot fungi. Biodiversitas 23: 4894-4905.* This study examines agricultural byproducts fermented with various rot fungi as a substrate of black soldier fly larvae. The experiment used a three-way randomized design with four replications. The first factor was substrate (cacao pod husk and oil palm frond), the second factor was fungi (*Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus sajor-caju*), and the third factor was environment condition (light and without light). Approximately 1200 of 7-day-old larvae were added to 600 g substrate treatment. The results showed that the solid-state fermentation substrates had the highest waste reduction parameters and level of consumption. Larvae in the experiment had a crude protein level of 38.84-58.88% of dry matter. Glutamic acid is the most abundant non-essential amino acid in larvae, while leucine is the most abundant essential amino acid. Solid-state fermentation substrate could improve the fatty acid profile quality of larvae in relation to high linoleic acid, polyunsaturated fatty acid, and unsaturated fatty acid percentages, especially CPH-pc substrate. The use of solid-state fermentation in larvae rearing decreased lignin in the cacao pod husk substrate. In conclusion, cacao pod husk substrate solid-state fermented with white rot fungi is not supported the optimum growth performance of BSFL but increases fatty acid profile quality.

Keywords: Agricultural byproduct, feed, fiber fraction, insect

INTRODUCTION

Black soldier fly larvae (BSFL) (Diptera: Statiomyidae, *Hermetia illucens*) are a potential food and feed product, in particular as a protein and fat source. This insect contains all of the essential amino acids required by humans and animals. BSFL meal at low inclusion levels (5%) may positively affect microbial populations and increase villi mucins (Biasato et al. 2020). Dietary oil extract of BSFL has been shown to improve feed conversion ratio in broiler chickens and did not harm organ weight or intestinal development (Kim et al. 2020). Meanwhile, partial substitution of soybean meal with BSFL in the diet of laying hens has been identified as safe and feasible (Bejaei and Cheng 2020). In addition, BSFL in a ruminant diet generally lowers methane emissions (in vitro study) and improves the growth rate of lamb and kids fed milk replacer containing BSFL meal (Jayanegara et al. 2017; Astuti and Wiryawan 2022). Interestingly, BSFL is also a safe product because it is not a disease vector (Popa and Green 2012) and produces antimicrobial peptides (Marusich et al. 2020).

Today, the main constraint to providing BSFL in feed is the quantity of substrate required for developmental rearing. Research studies have evaluated various organic byproducts as potential substrates for BSFL, such as coffee industry byproducts, vegetable waste, food waste, fish waste, and feces (Rehman et al. 2017; Jiang et al. 2019). Utilizing byproducts of agriculture as substrates can be the solution to providing continuous and inexpensive substrates. Meanwhile, BSFL has a superior ability to convert various organic byproducts. They can compensate for nutritionally poor and unbalanced diets in several ways, such as an increase in proteolytic activity (serin-proteases), an increase in the length of microvilli of midgut cells, creating a greater absorbing surface, and a decrease in α-amylase and lipase activity (Bonelli et al. 2020). On the other hand, abiotic factors such as direct sunlight are essential in matting, survival, growth, and development in the natural environment (Salam et al. 2022). The BSFL cultivation is influenced by environmental factors such as light (Kim et al. 2021). Therefore, artificial light sources are needed for indoor cultivation.

Several byproducts of food plants, including cacao pod husk (CPH) and oil palm frond (OPF), are available in very high quantities. The cultivation and processing of chocolate results in a cacao pod byproduct that includes placenta (2%) and husk (73.68%) (Puastuti and Susana 2014), a percentage that exceeds of the main product. In 2021, Indonesia produced around 703,600 tons/year of cacao (Badan Pusat Statistik 2022a). It means that Indonesia produced around 518,412 tons/year of cacao pod. The accumulation of CPH in the field leads to the growth of *Phytophthora palmivora* fungi, which can cause black pod disease. Another big cultivation in Indonesia is oil palm cultivation. Indonesia has 8,574,900-hectare oil palm land

(Badan Pusat Statistik 2022b). Oil palm cultivation results in OPF byproduct of around 47% of the whole crop (Kumneadklang et al. 2019), while not pruning fronds causes a decrease in production. A CPH and OPF are under-utilized but are used as forage for livestock with pre-treatment. These byproducts contain high levels of polysaccharides such as lignin, hemicellulose, and cellulose. Many researchers have therefore focused on enhancing their nutritional value. Solid-state fermentation (SSF) is one strategy for improving the nutritional value of high-polysaccharide byproducts and is a technology that is low energy, cost-efficient, and environmentally friendly (Kuttiyatveetil et al. 2019).

This study evaluates agricultural byproducts (CPH and OPF) fermented with various white rot fungi (*Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus sajor-caju*) under different environmental conditions (light and without light) as substrates for BSFL. The SSF method pre-treats the CPH and OPF using three fungal strains of white-rot fungi, which can produce enzymes able to break down polysaccharides. The fungus itself provides additional nutrient value to CPH and OPF as substrates. As a result of improvement in the nutritional value of the substrate, BSFL may be able to grow more efficiently.

MATERIALS AND METHODS

Materials

The CPH were donated by Kebun Pusat Penelitian Bioteknologi dan Bioindustri Indonesia (PPBBI), Bogor, Indonesia and OPF were collected from Cikabayan field, IPB University, Bogor, Indonesia. *Phanerochaete chrysosporium* (pc), *Trametes versicolor* (tv), and *Pleurotus sajor-caju* (ps) fungal strains from Indonesia Culture Collection, Cibinong, Bogor, Indonesia were used for SSF. Potato dextrose agar and potato dextrose broth from Merck were used as fungal media in this study.

Procedures

Fungal culture preparation

The fungus was grown on a sterile agar medium (4 mL) and incubated for seven days at room temperature (25°C-30°C). The fungal culture was transferred into a sterile broth medium (12 inoculating loops/250 mL) and incubated for four days (pc), six days (tv), and five days (ps) to prepare the fungal seed culture (each fungal has a different time of optimum growth).

Solid-state fermentation

The starter consisted of rice bran and molasses. The single strain fungi (250 mL of pc, tv, ps) was added to the starter and then adjusted to 70% (w/w) moisture using sterile soaked rice water and thoroughly mixed. The starter was incubated in a plastic bag (perforated for air circulation) at room temperature for seven days. The dried CPH and OPF substrates were ground using a grinder (sieve 1 mm). Samples of 60 g of substrate meal were placed in a plastic bag and adjusted to 70% (w/w) moisture content using sterile H_2O. Single strain starter fungi (120 mg of pc, tv, ps) was added, and the substrate was thoroughly mixed. The plastic bags were perforated with a sterile needle (perforated for air circulation). The plastic bags were then placed in an incubator with air circulation. The samples were incubated at room temperature for 21 days (Shrestha et al. 2008). Samples were collected at 21 days for nutrient composition analysis. The substrate meal was oven-dried at 60°C for 24 hours to inactivate the fungi (Gao et al. 2019).

Black soldier fly larvae rearing

The rearing method for BSFL was modified from Rehman et al. (2017). Approximately 11.8 g (1200 larvae) of 7-day-old BSFL were added to 600 g (as fed) substrate and placed into 27 cm x 20 cm x 9 cm plastic boxes (50 mg/larva/day). The substrate was kept moist, but no free water was present in the bottom of the boxes. The larvae were treated under two environmental conditions, there is cultivation with light and without light throughout of cultivation period. The artificial light (LED lamp) was used in this study for light conditions. To evaluate the efficiency of feeding of the BSFL on the substrate, BSFL growth was monitored during the rearing. Around 120 BSFL were weighed randomly every week and returned to the experiment after weighting. Nutrient intake, waste reduction rate, waste reduction index, conversion rate, and relative growth rate were analyzed in the final rearing using Equations 1, 2, 3, 4, and 5, respectively. When approximately 40%-50% of larvae had pre-pupated, the rearing was stopped. The BSFL separated from the substrate was oven-dried at 105°C for 10 min and at 60°C for two days. The substrate was dried at 60°C until a constant weight was achieved (Gao et al. 2019).

$$C\ (mg) = S - R\ (g)$$

$$\text{Nutrient intake (mg/larvae)} = (C\ (mg) \times \text{nutrient substrate (\%)}) / \text{total larvae} / 100$$

$$\text{Waste reduction rate (\%)} = (C / S) \times 100\%$$

$$\text{Waste reduction index (mg/day)} = C/t$$

$$\text{Conversion rate} = (Wlf - Wl0) / C \times 100\%$$

$$\text{Relative growth rate (mg/day)} = (Wlf - Wl0) / t$$

Where, C is total substrate consumption, S is total feed offered, R is total feed left over, t is rearing time, W10 is weight at the start of the process, and W1f is weight at the end of the process.

Nutrient composition analysis

Around 100 g of the substrates and BSFL were sampled for nutrient composition analysis. Determinations of moisture, organic matter, ash, crude protein, ether extract, crude fiber content were performed according to the AOAC official method (Association of Official Analytical Chemists 2005). Nitrogen-free extract content was calculated by the difference: Nitrogen-free extract (% DM) = 100-ash-crude protein-ether extract-crude fiber. Fiber

analysis was performed to determine neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose, lignin, and silica content according to the official Van Soest method (Van Soest et al. 1991). The BSFL was collected for amino acid and fatty acid profiling. Ultra-high-performance liquid chromatography performed on samples was used to determine the amino acid composition of protein-bound amino acids, following 18-5-17/MU/SMM-SIG(UPLC) procedure. Methionine content in larvae was estimated by a kinetic model (Miner et al. 2022). The fatty acid profiles of each sample were assessed by gas chromatography following procedure 18-6-1/MU/SMM-SIG(GC).

Data analysis

Three-ways ANOVA experimental design (Table 1) was used for the statistical analyses. All statistical analyses were performed using SAS On Demand for Academics. P-values of less than 0.05 were considered significant differences.

RESULTS AND DISCUSSION

Substrates reduction and growth performance of BSFL

The SSF substrates contained increased ash, ether extract, crude fiber, and decreased lignin content (Table 2). There were interactions between two factors, that is, substrate-fungi for the substrate reduction parameters (waste reduction rate and waste reduction index) (Table 3). The SSF-OPF substrates with all fungi had a higher waste reduction rate value than SSF-CPH substrates ($p<0.05$). Interaction was found between environmental condition-fungi for waste reduction rate and waste reduction index. The SSF-Pc fungi-without light substrate had the highest waste reduction rate and waste reduction index values ($p<0.05$). The three factors, a type of substrate, type of fungi, and environmental condition, obtained tangible interactions with conversion rate and relative growth rate values. Each treatment significantly impacted the further growth of BSFL. The BSFL reared on CPH-unfermented-light substrate had the highest conversion rate and relative growth rate values ($p<0.05$). The BSFL reared on SSF-OPH substrate had the lowest conversion rate and relative growth rate values ($p<0.05$). The biomass values in relation to rearing times are shown in Figures 1 and 2.

Nutrient intake of BSFL

There was an interaction between substrate-fungi and environmental condition-fungi on BSFL nutrient intake parameters (Table 4). Rearing on SSF substrate resulted in higher ash, crude protein, and crude fiber consumption than the unfermented substrate ($p<0.05$). The SSF-OPF substrate had the highest ash, crude protein, ether extract, crude fiber, and nitrogen-free extract consumption ($p<0.05$). The SSF-Pc fungi-without light substrate had the highest all-nutrient consumption ($p<0.05$).

Table 1. Three-ways ANOVA experimental design trial set up

Treatment	Substrate	Fungi	Environ. condition
CPH-light	CPH	unfermented	light
CPH-pc-light	CPH	Pc	light
CPH-tv-light	CPH	Tv	light
CPH-ps-light	CPH	Ps	light
CPH- without light	CPH	unfermented	without light
CPH-pc-without light	CPH	Pc	without light
CPH-tv-without light	CPH	Tv	without light
CPH-ps-without light	CPH	Ps	without light
OPF-light	OPF	unfermented	light
OPF-pc-light	OPF	Pc	light
OPF-tv-light	OPF	Tv	light
OPF-ps-light	OPF	Ps	light
OPF- without light	OPF	unfermented	without light
OPF-pc-without light	OPF	Pc	without light
OPF-tv-without light	OPF	Tv	without light
OPF-ps-without light	OPF	Ps	without light

Table 2. Nutrient composition of substrate

	CPH	CPH-pc	CPH-tv	CPH-ps	OPF	OPF-pc	OPF-tv	OPF-ps
Macro-nutrient composition of substrate								
Moisture (%)	11.84	37.89	17.35	39.93	10.40	35.06	12.52	35.22
Ash (% DM)	7.52	11.41	10.89	10.37	5.50	8.13	8.25	8.06
Crude protein (% DM)	10.50	11.37	10.64	11.30	8.76	8.75	8.72	9.23
Ether extract (% DM)	0.90	1.62	3.30	6.94	1.72	5.94	0.87	1.64
Crude fiber (% DM)	47.82	50.60	49.15	48.30	41.44	53.32	54.23	51.15
Nitrogen-free extract (% DM)	33.26	25.00	26.02	23.09	42.58	23.86	27.93	29.92
Fibre composition of the substrate (% DM)								
NDF	41.40	42.76	41.20	43.60	41.36	42.95	43.13	43.52
ADF	41.10	42.32	40.81	40.68	33.19	39.10	38.76	38.44
Hemicellulose	2.71	0.45	0.39	2.91	8.17	3.86	4.37	5.08
Cellulose	2.43	8.48	7.55	8.18	6.06	12.86	12.79	12.69
Lignin	38.64	33.56	32.89	32.38	26.66	24.79	24.31	24.60
Silica	0.03	0.03	0.37	0.12	0.47	1.45	1.66	1.15

Note: CPH: cacao pod husk; OPF: oil palm frond; pc: *Phanerochaete chrysosporium*; tv: *Trametes versicolor*; pc: *Pleurotus sajor-caju*; NDF: neutral detergent fiber; ADF: acid detergent fiber; DM: dry matter

Table 3. Substrate reduction and growth performance of BSFL

Treatment			Parameter			
Substrate	**Fungi**	**Environ. condition**	**Waste reduction rate (%)**	**Waste reduction index (mg/day)**	**Conversion rate (%)**	**Relative growth rate (mg/day)**
CPH	unfermented	Light	25.0 ± 4.27	3571.4 ± 609.90	26.9 ± 0.00^{a}	996.4 ± 48.82^{a}
CPH	pc	Light	20.2 ± 4.80	5057.3 ± 1199.73	7.8 ± 4.05^{cde}	387.5 ± 211.50^{bcde}
CPH	tv	Light	26.6 ± 7.41	6640.6 ± 1853.45	5.2 ± 1.55^{de}	364.6 ± 181.60^{cde}
CPH	ps	Light	23.3 ± 10.40	5812.5 ± 2599.13	8.7 ± 3.65^{cde}	463.5 ± 185.29^{bcde}
CPH	unfermented	Without light	25.8 ± 4.53	5327.6 ± 937.08	17.5 ± 3.60^{ab}	797.7 ± 117.66^{ab}
CPH	pc	Without light	32.7 ± 4.65	7538.5 ± 1073.48	6.2 ± 2.17^{de}	460.6 ± 150.06^{bcde}
CPH	tv	Without light	24.7 ± 10.71	5920.0 ± 2571.07	12.3 ± 3.50^{bcd}	696.0 ± 214.92^{abcd}
CPH	ps	Without light	24.7 ± 2.75	5933.3 ± 659.19	12.4 ± 1.89^{bcd}	707.0 ± 110.53^{abc}
OPF	unfermented	Light	16.7 ± 2.91	3584.8 ± 624.13	14.0 ± 6.21^{bc}	479.5 ± 151.00^{bcde}
OPF	pc	Light	33.2 ± 5.58	6419.4 ± 1080.12	4.0 ± 1.25^{e}	254.8 ± 92.41^{e}
OPF	tv	Light	39.4 ± 1.48	7883.3 ± 296.59	3.3 ± 2.04^{e}	260.8 ± 164.73^{e}
OPF	ps	Light	41.9 ± 1.58	8379.2 ± 316.63	3.2 ± 1.25^{e}	270.0 ± 112.71^{de}
OPF	unfermented	Without light	19.2 ± 2.72	4120.5 ± 582.37	18.7 ± 3.70^{ab}	756.3 ± 57.47^{abc}
OPF	pc	Without light	41.9 ± 0.53	8672.4 ± 109.95	6.3 ± 0.84^{cde}	548.3 ± 72.22^{abcde}
OPF	tv	Without light	39.7 ± 0.49	8206.9 ± 100.53	2.8 ± 0.96^{e}	229.3 ± 77.44^{e}
OPF	ps	Without light	41.0 ± 2.96	8487.1 ± 611.58	4.4 ± 2.91^{de}	387.1 ± 281.80^{bcde}
SEM			1.223	253.184	0.814	31.496
P-value						
Substrate			<0.001	<0.001	<0.001	<0.001
Fungi			<0.001	<0.001	<0.001	<0.001
Light			0.032	0.008	0.255	0.002
Substrate*Fungi			<0.001	0.006	0.127	0.068
Substrate*Light			0.840	0.867	0.236	0.530
Fungi*Light			0.014	0.021	0.134	0.607
Substrate*Fungi*Light			0.818	0.633	<0.001	0.005

Note: The superscript indicates significant difference (at least p<0.05); CPH: cacao pod husk; OPF: oil palm frond; pc: *Phanerochaete chrysosporium*; tv: *Trametes versicolor*; pc: *Pleurotus sajor-caju*

Table 4. Nutrient Intake of BSFL

Treatment			Parameter				
Subs-trate	**Fungi**	**Environ. condition**	**Ash (mg/larvae)**	**Crude protein (mg/larvae)**	**Ether extract (mg/larvae)**	**Crude fiber (mg/larvae)**	**Nitrogen-free extract (mg/larvae)**
CPH	unfermented	Light	9.4 ± 1.61	13.1 ± 2.24	1.1 ± 0.19	59.8 ± 10.21	41.6 ± 7.10
CPH	pc	Light	11.5 ± 2.74	11.5 ± 2.73	1.6 ± 0.39	51.2 ± 12.14	26.9 ± 6.39
CPH	tv	Light	14.5 ± 4.04	14.1 ± 3.94	4.4 ± 1.22	65.3 ± 18.22	34.6 ± 9.65
CPH	ps	Light	12.1 ± 5.39	13.1 ± 5.87	6.7 ± 2.88	56.1 ± 25.11	34.9 ± 15.61
CPH	unfermented	Without light	9.7 ± 1.70	13.5 ± 2.38	1.2 ± 0.20	61.6 ± 10.83	42.8 ± 7.53
CPH	pc	Without light	18.6 ± 2.65	18.6 ± 2.64	2.6 ± 0.38	82.6 ± 11.77	43.5 ± 6.19
CPH	tv	Without light	13.4 ± 5.83	13.1 ± 5.70	4.1 ± 1.77	60.6 ± 26.33	37.8 ± 9.79
CPH	ps	Without light	10.8 ± 4.26	14.0 ± 1.55	7.2 ± 2.85	50.2 ± 19.84	37.1 ± 4.12
OPF	unfermented	Light	4.6 ± 0.80	7.3 ± 1.28	1.4 ± 0.25	34.7 ± 6.03	35.6 ± 6.20
OPF	pc	Light	13.5 ± 2.27	14.5 ± 2.44	9.9 ± 1.66	88.4 ± 14.88	49.4 ± 8.32
OPF	tv	Light	16.3 ± 0.61	17.2 ± 0.65	1.7 ± 0.06	106.9 ± 4.02	56.8 ± 2.14
OPF	ps	Light	16.9 ± 0.64	19.3 ± 0.73	3.4 ± 0.13	107.1 ± 4.05	66.1 ± 2.50
OPF	unfermented	Without light	5.3 ± 0.75	8.4 ± 1.19	1.7 ± 0.23	39.8 ± 5.63	40/9 ± 5.79
OPF	pc	Without light	17.0 ± 0.22	18.3 ± 0.23	1.2 ± 0.20	111.7 ± 1.42	62.5 ± 0.79
OPF	tv	Without light	16.4 ± 0.20	17.3 ± 0.21	1.7 ± 0.02	107.6 ± 1.32	57.1 ± 0.70
OPF	ps	Without light	16.5 ± 1.19	18.9 ± 1.36	3.4 ± 0.24	104.9 ± 7.56	64.7 ± 4.66
SEM			0.595	0.540	0.356	3.578	1.707
P-value							
Substrate			0.255	0.074	0.914	<0.001	<0.001
Fungi			<0.001	<0.001	<0.001	<0.001	0.002
Light			0.114	0.039	0.472	0.074	0.007
Substrate*Fungi			<0.001	<0.001	<0.001	<0.001	<0.001
Substrate*Light			0.850	0.638	0.972	0.874	0.685
Fungi*Light			0.011	0.018	0.782	0.007	0.026
Substrate*Fungi*Light			0.608	0.673	0.860	0.890	0.846

Note: The superscript indicates significant difference (at least p<0.05); CPH: cacao pod husk; OPF: oil palm frond; pc: *Phanerochaete chrysosporium*; tv: *Trametes versicolor*; pc: *Pleurotus sajor-caju*

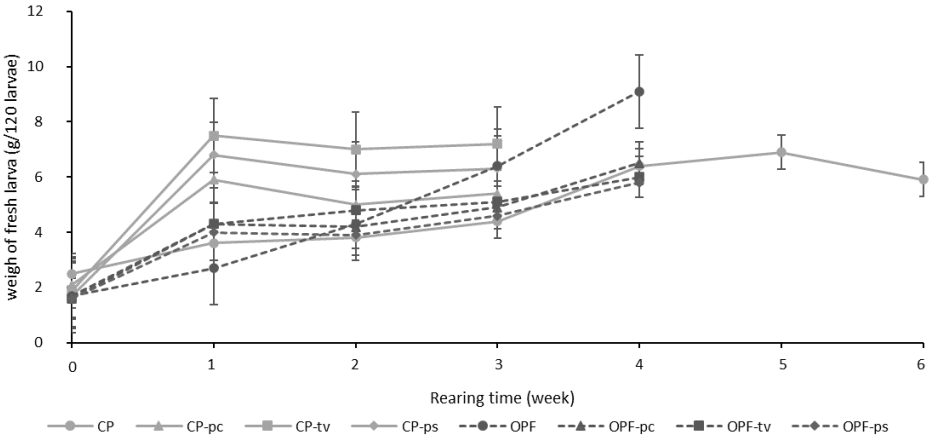

Figure 1. Black soldier fly larvae weight gained fed various substrates with light condition

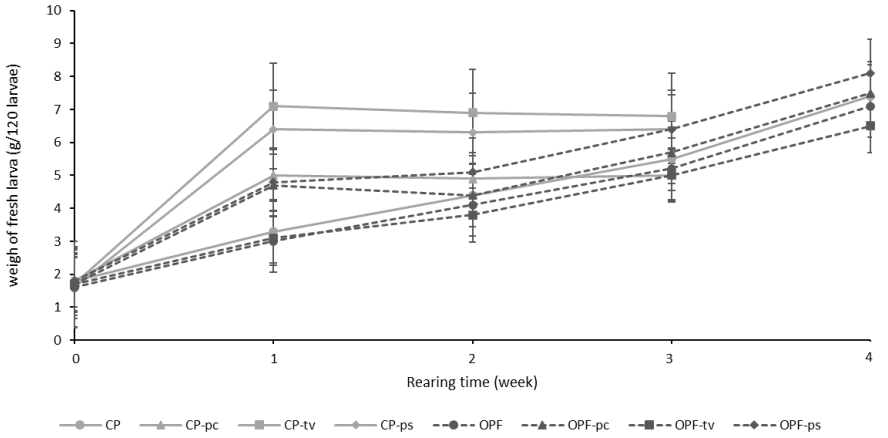

Figure 2. Black soldier fly larvae weight gained fed various substrates without light condition

Nutrient composition of BSFL

BSFL in this study had nutrient content as follows: crude protein: 38.84-58.88% DM; ash: 18.14-32.73% DM; ether extract: 1.43-12.04% DM; carbohydrate: 8.62-9.99% DM; energy from fat: 2.34-21.06 kcal/100 g, and total energy: 51.48-116.76 kcal/100 g (Table 5). The results showed that there was a trend for non-essential amino acids to have higher values in apparently random data than essential amino acids (Table 6). BSFL reared on SSF with light conditions had a pattern of higher values of all amino acids than those reared on unfermented substrates. BSFL reared without light showed no pattern in the data. Lauric acid (C12: 0) was the most abundant fatty acid in BSFL in all kinds of substrate treatments (Table 7). There was a trend for linoleic acid (C18: 3), polyunsaturated fatty acid (PUFA), and unsaturated fatty acid (UFA) to have higher values in SFF substrates. In contrast, SSF substrates produced lower C12: 0 and saturated fatty acid (SFA) than unfermented substrates.

Nutrient composition of the left-over substrate (frass)

The ash and crude protein content of left-over substrate (frass) from all treatment samples increased. The ash content of CPH frass was increased to 1.0%-4.2%, while OPF frass was increased to 0.7%-2.4%. The crude protein content for CPH frass was increased to 3.0%-5.8%, while OPF frass increased to 0.1%-3.2%. The CPH frass from with light rearing, both SSF and unfermented, had higher crude protein content than without light rearing, while the OPF frass had various data. The ether extract level increased slightly except in CPH-ps fungi frass and OPF-pc fungi frass. The crude fiber and nitrogen-free extract of all treatments had various graphic patterns (Figure 3).

The NDF was increased except in the CPH-pc fungi frass (with and without light) because of decreasing cell content percentage for BSFL development. The ADF data was varied because of the changing nutrient component of ADF. Hemicellulose was increased except in OPF frass (with or without light). The cellulose content of the frass from all treatments provided varied data. Almost all treatment frass had a reduced lignin content, except that from OPF-without light frass. The silica content of frass was increased, except in CPH-pc fungi without light frass (Figure 4).

Table 5. Nutrient composition of black soldier fly larvae

Substrate	Crude protein (% DM)	Ash (% DM)	Ether extract (% DM)	Carbohydrate (% DM)	Energy from fat (kcal/100 g)	Total energy (kcal/100 g)
Light						
CPH	53.29	30.21	5.39	11.11	7.29	46.01
CPH-pc	45.80	24.81	3.73	25.66	9.90	94.22
CPH-tv	47.15	26.58	5.62	20.65	17.64	112.20
CPH-ps	48.16	24.61	5.16	22.06	16.56	116.76
OPF	43.98	24.14	12.04	19.84	17.10	57.38
OPF-pc	45.30	18.14	2.88	33.67	4.86	64.06
OPF-tv	45.82	26.95	3.45	23.78	6.39	63.63
OPF-ps	50.34	32.73	3.30	13.63	5.76	55.32
Without light						
CPH	58.88	24.87	10.59	8.62	10.44	51.48
CPH-pc	48.59	23.73	3.90	23.79	6.21	57.45
CPH-tv	49.45	24.58	3.62	22.34	11.34	111.34
CPH-ps	53.17	25.84	7.41	13.58	21.06	105.38
OPF	46.63	22.88	4.13	26.38	5.94	52.66
OPF-pc	38.84	19.75	1.43	39.99	2.34	59.82
OPF-tv	56.42	18.88	3.88	20.82	6.48	63.76
OPF-ps	44.98	22.68	4.19	28.16	7.02	61.46

Note: CPH: cacao pod husk; OPF: oil palm frond; pc: *Phanerochaete chrysosporium*; tv: *Trametes versicolor*; pc: *Pleurotus sajor-caju*; DM: dry matter

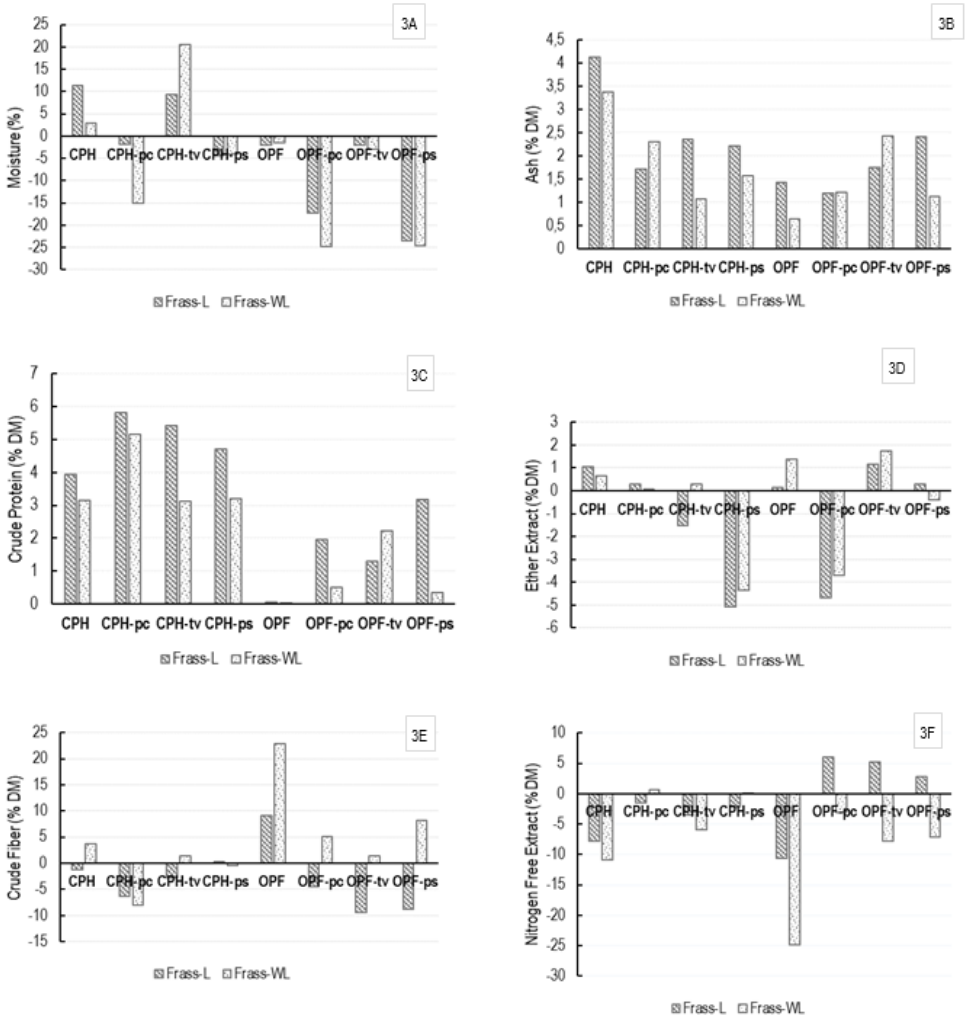

Figure 3. Makro-nutrient dynamics chart of frass-L (frass of BSFL reared with light) and frass-WL (frass of BSFL reared without light)

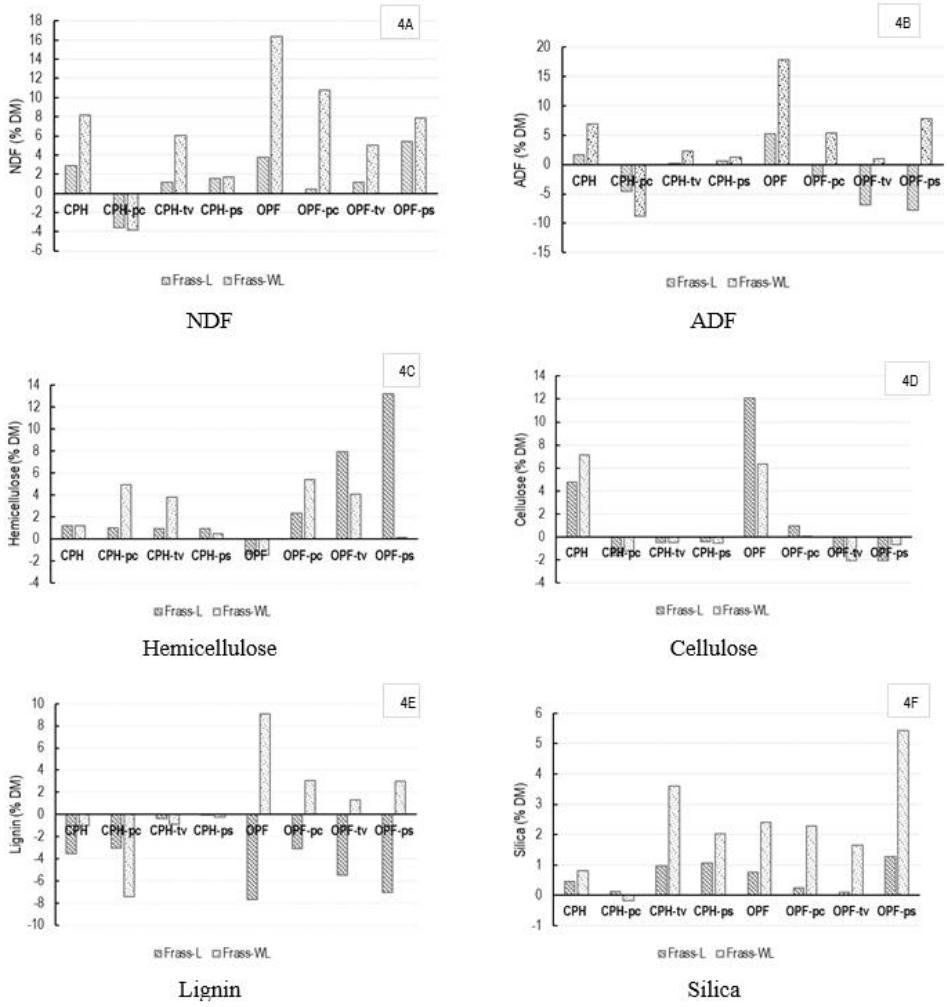

Figure 4. Fibre fraction dynamics chart of frass-L (frass of BSFL reared with light) and frass-WL (frass of BSFL reared without light)

Discussion

Our findings revealed that BSFL readily fed and grew on all treatment substrates. Our results agree with previous reports that the performance parameters of BSFL are significantly influenced by substrate treatment (Spranghers et al. 2017; Barragan-Fonseca et al. 2018; Chia et al. 2020). The SSF substrates significantly affected all recorded growth parameters (waste reduction rate, waste reduction index, conversion rate, relative growth rate). The significant interaction between substrate-fungi or environmental condition-fungi in waste reduction rate and waste reduction index reveals that the main effect of substrate on the growth performance of BSFL depends on the fungi strain used for SSF. The SSF-OPF had a high waste reduction rate value, meaning BSFL can reduce more substrate than SSF-CPH and unfermented substrates. The lower consumption of CPH could result from the highly complex polymer present in lignin cellulose in CPH, providing protection that prevents BSFL from hydrolyzing the nutrient. Another study reported that microbes in BSFL gut and excretions could hydrolyze fibers, thus making nutrients available for BSFL development (Gold et al. 2018; Palma et al. 2020). However, in the present study, hydrolyzation was not optimum, even though the BSFL gut contains microbes that produce cellulase (Kim et al. 2011; Lee et al. 2014; Manurung et al. 2016). The waste reduction rate and waste reduction index values increased when the substrate was supplemented with fungi as an SSF treatment. The SSF treatment may decrease structurally complex lignocellulose and release cellulose that is available for digestion by BSFL. The relatively high conversion rate and relative growth rate of BSFL reared on the unfermented substrate indicate that the nutrients from the substrate could be assimilated to build BSFL biomass. BSFL development in each treatment could also have been restrained by excessive fungal growth on the substrate (Tschirner and Simon 2015). The lower conversion rate and relative growth rate value of the BSFL reared on the SSF treatment might be a sign that protein supply from fungi in the SSF treatment was sufficient for BSFL development but that in these conditions, BSFL prioritizes energy allocation for metabolism rather than growth.

Table 6. Total detected amino acid content of black soldier fly larvae

Amino acids (g/kg DM)	Substrates															
	Light								Without light							
	CPH	CPH-pc	CPH-tv	CPH-ps	OPF	OPF-pc	OPF-tv	OPF-ps	CPH	CPH-pc	CPH-tv	CPH-ps	OPF	OPF-pc	OPF-tv	OPF-ps
Essential amino acids																
Histidine	8.1	9.5	10.2	11.6	10.2	7.4	8.8	7.2	11.0	7.3	12.2	10.9	6.9	6.5	7.4	7.8
Isoleucine	14.2	15.9	15.8	19.7	16.9	13.0	13.7	14.3	16.1	14.4	18.8	19.0	15.5	12.6	14.3	14.4
Leucine	21.5	25.0	24.8	30.8	26.9	20.5	22.1	22.9	25.4	22.2	29.2	29.8	24.3	19.7	22.7	23.4
Lysine	16.7	20.4	19.4	25.4	18.7	16.0	15.7	18.6	17.7	19.2	23.3	23.9	22.8	16.6	17.5	18.3
Methionine*	19.8	15.6	15.6	15.6	16.6	17.3	16.6	16.6	16.6	16.1	15.9	15.9	16.6	16.6	16.6	16.6
Phenylalanine	15.3	13.6	15.0	18.0	16.5	13.0	13.2	11.5	19.4	11.0	21.1	18.5	11.6	10.1	12.4	11.7
Threonine	16.6	18.4	17.9	23.1	20.5	15.8	16.4	15.6	20.2	15.5	23.1	22.3	16.2	13.9	16.0	16.2
Valine	21.5	24.9	24.7	29.9	27.7	20.8	23.1	23.0	26.8	22.8	28.6	29.4	23.0	20.3	24.4	26.3
Non-essential amino acids																
Alanine	20.8	27.7	27.8	31.1	29.1	21.5	25.6	25.2	27.7	26.1	32.0	32.1	27.6	22.6	25.8	27.6
Arginine	16.2	18.6	17.9	23.1	20.7	19.0	16.9	15.9	22.0	14.8	23.7	22.2	15.7	13.4	16.3	15.9
Aspartic acid	27.9	28.8	33.2	36.1	29.6	25.1	26.4	27.1	30.4	28.3	35.4	35.6	28.9	23.7	26.2	25.8
Glutamic acid	39.0	46.4	47.5	52.8	44.2	37.0	38.5	40.7	43.1	41.8	51.6	51.2	42.8	35.8	39.0	39.3
Glycine	24.9	30.0	29.1	35.9	28.4	23.1	26.3	24.4	29.8	24.6	31.9	31.7	22.4	21.1	23.5	26.0
Proline	15.9	18.7	19.3	22.3	20.2	14.5	17.2	17.0	19.6	17.5	20.8	21.6	17.9	14.7	16.4	19.0
Serine	15.7	18.2	17.8	22.0	21.1	15.0	16.6	15.4	19.6	15.3	21.6	21.5	16.0	13.5	15.5	16.6
Tyrosine	17.1	17.2	18.5	23.0	21.9	13.9	17.4	12.9	23.6	12.3	24.4	21.8	14.7	11.5	13.6	15.6

Note: *: Estimated by a kinetic model (Miner et al. 2022), CPH: cacao pod husk, OPF: oil palm frond, pc: *Phanerochaete chrysosporium*, tv: *Trametes versicolor*, pc: *Pleurotus sajor-caju*

Table 7. Total detected fatty acid content of black soldier fly larvae

(% Total fatty acid, DM)	Substrates															
	Light								Without light							
	CPH	CPH-pc	CPH-tv	CPH-ps	OPF	OPF-pc	OPF-tv	OPF-ps	CPH	CPH-pc	CPH-tv	CPH-ps	OPF	OPF-pc	OPF-tv	OPF-ps
C 10: 0	0.88	0.26	0.25	1.20	0.42	0.19	0.14	0.11	0.83	0.25	0.17	0.21	0.53	nd	0.26	0.11
C 12: 0	12.43	4.08	4.36	9.14	18.71	2.25	1.66	1.89	14.90	2.70	3.24	3.04	17.35	1.74	2.91	1.74
C 14: 0	0.00	0.00	0.06	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C 15: 0	0.54	0.47	0.66	0.56	0.30	0.63	0.53	0.69	0.42	0.37	0.60	0.72	0.39	0.60	0.58	0.70
C 16: 0	7.05	6.13	8.42	6.33	5.71	8.38	6.81	6.48	6.43	7.50	7.18	7.87	5.84	7.56	7.87	9.07
C 17: 0	0.59	0.71	1.09	0.71	0.27	0.79	0.81	0.85	0.48	0.57	0.98	1.21	0.34	0.77	0.85	0.98
C 18: 0	2.52	2.19	2.03	1.83	1.50	2.51	2.06	1.69	2.45	2.32	2.26	2.49	2.01	2.89	2.58	2.10
C 14: 1	nd	nd	0.06	nd	0.12	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C 16: 1	1.86	2.46	4.89	3.52	2.18	1.88	1.71	2.06	1.73	1.62	3.99	3.40	2.10	1.54	1.88	2.18
C 17: 1	0.45	0.59	0.81	0.57	0.38	0.42	0.37	0.42	0.34	0.33	0.83	0.70	0.43	0.33	0.45	0.45
C 18: 1 W9C	5.26	6.12	4.57	4.35	4.71	6.74	6.82	7.00	4.90	6.97	5.68	5.67	4.84	7.69	7.08	6.61
C 18: 2 W6C	1.02	3.18	1.85	2.11	0.69	2.99	4.81	4.27	1.03	3.58	2.33	2.47	0.88	3.41	3.32	3.33
C 18: 3	0.21	0.47	0.41	0.38	0.10	nd	0.19	0.13	0.18	0.19	0.28	0.34	nd	nd	nd	0.18
C 20: 4 w6	0.37	0.57	0.77	0.29	0.18	0.84	0.32	0.67	0.31	0.38	0.71	0.58	0.27	0.55	0.47	0.96
C 20: 5 w3	0.29	0.61	0.48	0.30	0.12	0.63	0.31	0.40	0.20	0.49	0.62	0.51	0.19	0.49	0.36	0.33
MUFA	8.03	10.02	11.46	9.21	7.55	9.57	8.90	9.48	7.34	9.66	11.47	10.77	7.45	9.55	9.41	9.23
PUFA	1.89	4.82	3.52	3.09	1.09	4.47	5.63	5.46	1.73	4.64	3.94	3.89	1.47	4.57	4.15	4.79
Saturated fat	27.53	15.20	18.54	23.07	30.93	16.24	13.37	13.07	29.30	15.15	16.09	17.20	30.11	15.25	16.82	15.98
Unsaturated fat	9.91	14.84	14.98	12.29	8.64	14.04	14.53	14.95	9.07	14.30	15.42	14.67	8.92	14.13	13.56	14.02

Note: CPH: cacao pod husk; OPF: oil palm frond; pc: *Phanerochaete chrysosporium*; tv: *Trametes versicolor*; pc: *Pleurotus sajor-caju*; MUFA: monounsaturated fatty acid; PUFA: poly-unsaturated fatty acid; DM: dry matter, nd: not detected

Considering suitable artificial light sources is a high priority because mating, survival, growth, and development depend on direct sunlight (Zhang et al. 2010; Klüber et al. 2020; Salam et al. 2022). In contrast, light is an obstacle to the movement of BSFL. In the present study, LED light did not influence growth performance in the unfermented substrate. The differences resulting from the light condition were found in the SSF-CPH treatment, reflecting that degradation of the substrate and growth of BSFL was low in the light condition. This is related to BSFL being inclined to move away from light.

The development time of each substrate in the rearing of BSFL to the prepupa stage was investigated in this study. Findings are that a high average BSFL body weight was observed for the OPF substrate. The various development times were influenced by the substrate composition and the ratio of nitrogen-free extract and protein. Even though CPH has a high level of nitrogen-free extract (starch and glucose) and protein, the presence of lignin that may chelate with carbon complex polymers leads to low degradable effects. Protein and nitrogen-free extract ratios are the primary nutrient factors that can significantly impact the growth of BSFL (Tinder et al. 2017). Both are macronutrients that require ATP for glycolysis and are then converted into triglycerides in the Krebs cycle before being stored as lipids (Noreika et al. 2016). In the present study, we found greater intake in BSFL grown on the SSF substrate than on the unfermented substrate. The present study found that nitrogen-free extract had the highest consumption level, followed by crude fiber, crude protein, ash, and ether extract. Findings have suggested that BSFL could degrade some nutrients because of the intense activity of enzymes such as amylase, protease, and lipase in the intestinal gut of BSFL (Kim et al. 2011). Previous studies have shown the presence of a cellulase gene in the intestinal microbiota of BSFL that can help increase the available nutrients for BSFL development (Nguyen et al. 2013; Palma et al. 2019). However, the development growth time of BSFL is slower when diet is not favorable or the substrate is unbalanced, BSFL may experience metabolic costs, reduce their substrate intake, and prolong their feeding time (Diener et al. 2009). Food deprivation can eventually lead to death, but this situation was not seen in this study.

Managing high-fiber lignocellulose byproduct substrates for the cultivation of BSFL was one of the challenges addressed by this study. Cellulose is harder to digest and remains in the spent substrate in the ash portion. The lignocellulose structure is micronized by BSFL mastication and this changes the amorphous and crystalline cellulose ratio. Crystalline cellulose should be more susceptible to enzyme hydrolysis than amorphous cellulose (Howdeshell and Tanaka 2018). Therefore, SSF as a treatment for unchanged relative cellulose ratio of fiber lignocellulose in CPH and OPF is needed. The high-value fiber intake results from a higher percentage of fiber in the substrate than other nutrients. OPF and CPH have pattern differences in their fiber structure that can lead to absorption differences by BSFL.

After the rearing period, differences between the experimental substrates existed. The highest crude protein content was in BSFL reared on unfermented CPH. The crude protein content of BSFL from different substrates generally reflects the crude protein content of the substrate used (Nyakeri et al. 2017; Adebayo et al. 2021). Meanwhile, the crude protein content of BSFL in this study was slightly higher than those raised on various other substrates except for chicken feed, brewery waste, food remains, and fruit waste, as reported by Adebayo et al. (2021). The ash level in the BSFL reflects the length of rearing time but does not contribute to building BSFL biomass (Kuttiyatveetil et al. 2019). A more extended time of rearing would produce more ash content because of the development of the exoskeleton. Ash content of BSFL increases from 0 to 29 days (Do et al. 2020; Hoc et al. 2020). BSFL reared on SSF substrate had lower ether extract than BSFL reared on the unfermented substrate. Nonetheless, a previous study reported that supplementation with BSFL in feed for broilers and hens could harm carcass fat content, in that although there was no sign of an impact on the total fat content of broiler carcasses, there was deterioration in the fatty acid profile (increasing SFA percentage) (Cullere et al. 2019). Supplementation of BSFL in hens' diets could be implicated in increasing yolk percentage due to increased yolk fat (Bejaei and Cheng 2020). Carbohydrate content reflects non-fiber carbohydrates such as starch and glucose. The BSFL fed on SSF substrate had high carbohydrate and total energy content. Meanwhile, the energy from fat in the BSFL fed on experimental substrates presented with various values due to variation in fat mechanisms in the BSFL body (Hoc et al. 2020). At first glance, there is a high positive correlation between the ether extract content of the BSFL and the energy acquired from fat content. Moreover, the ether extract content of the BSFL was influenced by the nitrogen-free extract content of the substrate. Nitrogen-free extract can transform into several fatty acids because of enzymes that support the transformation mechanism (Hoc et al. 2020).

A similar pattern is reported in the literature for most amino acids in BSFL (Spranghers et al. 2017; Lalander et al. 2019). The dominant essential amino acids present in our study were leucine and valine, while glutamic acid was the most abundant non-essential amino acid. Meanwhile, the value of lysine and methionine could be used to assess the limiting amino acids. Lysine was higher in BSFL fed on the CPH-SSF substrate than in the CPH-unfermented substrate. In contrast, lysine in BSFL fed on OPH-SSF fluctuated but over a small range. Methionine was higher in BSFL fed on the CPH-unfermented than CPH-SSF substrate. On the other hand, methionine in BSFL fed on OPF-unfermented and OPF-SSF similar tend to be the same. These are essential differences because lysine and methionine are the limiting amino acids in poultry and aquatic animals (Fischer et al. 2021). In addition, minor differences were observed for all amino acids in BSFL reared in the experimental substrates, suggesting that the substrate had no substantial influence on the amino acid

profiles of BSFL (Spranghers et al. 2017; Lalander et al. 2019).

Our study confirmed a previous report that indicated most BSFL as containing a high percentage of saturated fatty acid (SFA), followed by monounsaturated fatty acid (MUFA) and PUFA (Ewald et al. 2020). The results were lower than in some recent studies in which BSFL contained SFA of up to 76%, MUFA of up to 32%, and PUFA of up to 23% (Spranghers et al. 2017; Ewald et al. 2020; Saadoun et al. 2020). Meanwhile, other studies report that the content of the profile of fatty acids in BSFL will tend to be smaller if they are reared on vegetable and fruit waste (Meneguz et al. 2018; Giannetto et al. 2020). This suggests that substrates with high fiber levels would be linked with low fatty acid profile values. Comparing the nutritional fatty acid profiles of BSFL as feed, the SSF treatment produced lower SFA and higher PUFA content than the unfermented substrate. The fatty acid profile of BSFL is rich in medium-chain fatty acids, especially C12: 0. A C12: 0 can be advantageous in gut health and is implicated in good growth performance (Zeitz et al. 2015). A C12: 0 content was strongly decreased in BSFL reared on the SFF treatments (by up to 10%). However, the low C12: 0 content and other fatty acid profiles may improve feed storability and increase protein digestibility (Schiavone et al. 2018). Our study also found that substrates with SSF pre-treatment are capable of improving the quality of fatty acid profiles because they are rich in PUFA.

Predominantly, BSFL degraded and absorbed main cell structures such as protein and starch, then degraded and partially absorbed cell walls. It should be noted that during BSFL rearing, the overall frass weight kept reducing. Some macronutrients (protein, ash, and to a small extent, ether extract) of frass were increased because of input of nutrients from fungi in the substrate of BSFL. The various percentages of fiber and nitrogen-free extract would be impacted by the range of changing percentages of the other nutrients in frass. Our study report that BSFL rearing combined with SSF decreased lignin in CPH but increased lignin in OPF. BSFL has two major digestion processes: the mouthparts necessary for food processing and ingestion and intestinal enzymes (Shelomi et al. 2020). A previous study showed substrate significantly influenced the microbial community in the gut of BSFL (Spranghers et al. 2017). Interestingly, a stable core group of bacteria can be present within the BSFL to guarantee dietary flexibility. The core community was present in at least 80% of total bacteria. These core bacteria are *Actinomyces* sp., *Dysgonomonas* sp., and *Enterococcus* sp. (Dietrich et al. 2014; Klammsteiner et al. 2020). *Dysgonomonas* sp. was abundant in the gut of BSFL and is chiefly known for its crucial role in the degradation of recalcitrant lignocellulose (Bruno et al. 2019; Klammsteiner et al. 2020). This explains how the BSFL can reduce lignin in the substrate. However, adverse results were found in the form of increasing lignin in the OPF substrate. It may be suspected that this is because of the reactivation of fungi in the substrate. The time is also influenced by the amount and structure of media or substrate (Zuleta-Correa et al. 2016). Lignin increase in OPF was thought to be due to non-optimum development time and the fact that BSFL digests its nutrients simultaneously.

In conclusion, BSFL had a shorter growth time in CPH than OPF, of around one week. SSF treatment with *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus sajor-caju* can not support the optimum growth performance of BSFL but increase fatty acid profile quality because of higher PUFA percentage. After bioconversion by BSFL, the frass component changed. Interestingly, BSFL can reduce lignin content in CPH.

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