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Analyzing the Nutritional Value of *Protaetia brevitarsis Larvae* Feeding on Coffee and Oyster Mushroom Cultivation By-products

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Abstract

This study aimed to validate the nutritional superiority and safety of fermented coffee by-products (CB) and fermented oyster mushroom cultivation by-products (OMCB) as alternative food sources for *Protaetia brevitarsis* larvae. Thus, we conducted a comparative analysis of the nutrient composition of the food sources and developmental characteristics of the larvae. *P. brevitarsis* larvae have traditionally utilized various by-products as food sources and are able to efficiently utilize these. The analysis of the nutrient composition of the food sources indicated that, in comparison to control group fermented oak sawdust (FOS), the content of crude protein was 2.2 folds higher in larvae fed with OMCB and 3.2 folds higher in larvae fed with fermented 50% CB + 50% OMCB (CB + OMCB). Moreover, the cellulose content, utilized as a nutritional source for the larvae, was 1.3 folds higher in the CB + OMCB group than in the control group. Significantly, the weight of larvae fed with OMCB and CB + OMCB increased 1.7–4.2 folds compared to those fed with FOS. Additionally, the survival rate of larvae before the formation of pupal cells was over 90% in all groups. Therefore, it was concluded that CB and OMCB contain various nutrients without harmful composition and have a larval growth-promoting effect. Consequently, they are considered appropriate dietary materials for *P. brevitarsis* larvae. This study enhances our understanding of by-product usages by *P. brevitarsis* larvae and confirms their potential as sustainable food resources.

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Protaetia brevitarsis, coffee by-products, oyster mushroom cultivation by-products, food ingredients

Introduction

Protaetia brevitarsis has been used as a traditional herbal remedy for liver diseases for centuries. Recently, it has garnered significant attention as an insect resource for exploring and developing useful bioactive substances (Queiroz *et al*., 2023). In studies conducted using a diethylnitrosamine-induced hepatotoxic mouse model, administration of freeze-dried *P. brevitarsis* larval powder reduced liver damage (Lee *et al*., 2014). Research on thrombolytic enzymes and their protective effects against carbon tetrachloride-induced liver toxicity in rats has further validated their therapeutic potential (Chon *et al*.,

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2012). As research on functional health foods progresses, the anticipated increase in demand for *P. brevitarsis* necessitates the development of cost-effective alternative feeds (D'Antonio *et al*., 2023). Currently, a significant portion of the feed for this species larvae is sourced from oak sawdust, which is purchased externally, complicating the efforts to reduce costs. Mass production has also led to the destruction of oak trees. Moreover, concerns are raised owing to oak-sawdust shortage and waste-wood use, which is unsuitable for consumption (Kim *et al*., 2022). The nutritional composition of edible insects can vary even within the same species, depending on the habitat and environmental conditions, as well as the diet consumed by the insects (Ham *et al*., 2021; Oliveira *et al*., 2024). Thus, there is a need to enhance the nutritional value of insects by feeding them recyclable agricultural by-products and food processing residuals (Queiroz *et al*., 2023; Song *et al*., 2019). Such an approach not only optimizes the nutritional profile of insects as food ingredients but also contributes to sustainable agricultural practices by reducing waste (Vinci *et al*., 2022). This strategy aligns with the broader goals of sustainability and resource efficiency and underlines the importance of tailoring insect diets to improve their potential as viable food sources (Aguilar-Toalá *et al*., 2022). Therefore, there is an urgent need to develop new foods that address both nutritional value and safety concerns regarding heavy metals.

Coffee by-products (CB), obtained from the coffee extraction process, contain various nutrients, including dietary fiber and protein, and exhibit antioxidant properties (Esquivel and Jiménez, 2012; Janissen and Huynh, 2018; Stylianou *et al*., 2018). Owing to these features, coffee grounds are widely used in the food and pharmaceutical sectors (Ballesteros *et al*., 2014; Cruz *et al*., 2012; Singh *et al*., 2023). Furthermore, research is ongoing on their application as a resource for developing seed-added pellets, packaging materials, and substrates for biofuel production (Díaz-Jiménez and Moya, 2022; Garcia and Kim, 2021; Soares *et al*., 2015). Recent studies utilizing coffee grounds as a food source for *Hermetia illucens* larvae have demonstrated significant bioconversion efficiency (Fischer *et al*., 2021; Khaekratoke *et al*., 2022; Romano *et al*., 2022). Coffee grounds are recycled across diverse sectors, including the food, agriculture, energy, cosmetics, and environmental industries, positioning them as valuable sustainable resources (Blinová *et al*., 2017; Zengin *et al*., 2020). Approximately 14 million tons of mushrooms valued at around 50 billion dollars are produced worldwide annually (Łysakowska *et al*., 2023). Spent mushroom substrates (SMS) are recycled in several sectors, including new mushroom cultivation media and bio-fertilizers and enzyme production, following the principles of a circular economy (Leong *et al*., 2022; Martín *et al*., 2023; Zied *et al*., 2020). SMS positively affect growth and have been certified as safe alternative food source for poultry, ruminants, and insects (Baptista *et al*., 2023; Foluke *et al*., 2014; Kim *et al*., 2011). Upon evaluation as an insect food source for *Tenebrio molitor* larvae, SMS have demonstrated high nutritional value (Li *et al*., 2019). However, despite research on the use of SMS as a food source for *P. brevitarsis* larvae, studies on its nutritional effects and larval development remain insufficient (Lee *et al*., 2018; Li *et al*., 2020; Wei *et al*., 2020).

To investigate the nutritional efficacy and safety of CB and oyster mushroom cultivation by-products (OMCB) as substitute nourishments for *P. brevitarsis*, we conducted a comparative examination of the nutrient profiles of these alternative foods and the corresponding developmental attributes of the larvae.

Materials and Methods

Experimental Insects and Rearing

P. brevitarsis was reared in an insectarium at the National Institute of Agricultural Sciences, Industrial Insect and Sericulture Division. The insects were maintained at a temperature of 25°C, with relative humidity of 50–60% and a photoperiod of 16D:8D. The primary food source for fermented oak sawdust (FOS) was procured from Healing Bugs in Gimje. *P. brevitarsis* larvae were reared in rectangular plastic rearing boxes (27 cm length × 36 cm width × 8 cm height) with 30 individuals per box across three boxes for approximately 60 days.

Development Characteristics of *P. brevitarsis* Larvae

Preparation of Food Sources

The OMCB used in the experiment were discarded after growing the oyster mushrooms. These were purchased from the Chain Mushroom Farm in Hwaseong and the Gyeryongsan Oyster Mushroom Farm in Gongju. Initially, the substrates were prepared by mixing 50% ground OMCB and 50% CB and, then, by adding approximately 65% water. Subsequently, the mixture was divided into breathable burlap bags in quantities of 30–40 liters and fermented in a sealed

fermentation room. The humidity of the fermentation room was maintained at approximately 60%, and the fermentation period was maintained for six weeks.

Growth of P. brevitarsis Larvae on the Different Food Sources

We conducted the experiment using three plastic rearing boxes (25.7 cm length × 16.9 cm width × 14.0 cm height), designated as FOS, with 100% OMCB and 50% CB + 50% OMCB, each containing 30 larvae per box fed with 300 g of fermented sawdust per feeding, replicated thrice. The rearing conditions for the larvae included a temperature of 25°C, relative humidity of 60%, and sawdust moisture content of 60%. To investigate the larvae's developmental characteristics, we monitored their weekly survival rates and weights.

Nutritional Analysis of *P. brevitarsis*

Preparation of P. brevitarsis for Analysis

The *P. brevitarsis* larvae were reared separately to compare and analyze the nutritional composition and harmful substances from different food sources provided to them. A total of 4 kg each of FOS (the standard food source for *P. brevitarsis* larvae), OMCB, and CB + OMCB were prepared and used to rear 250 larvae in plastic rearing boxes (54 cm length × 40.5 cm width × 18.5 cm height) in three replicates. The rearing environment for the larvae was maintained at a temperature of 25°C, relative humidity of 50–60%, and a moisture content of 50–60% in the sawdust. On reaching the harvest weight (2.5 g per larva), the larvae were fed with glutinous rice for 2 days and then starved for 2 days. After starvation, the larvae were freeze-dried, and the nutritional composition and harmful substance content were measured.

Analysis of General Composition

A general component analysis was conducted according to the standards set by the Association of Official Analytical Chemists (AOAC) (Baur and Ensminger, 1977). Moisture content was determined by using an atmospheric pressure drying method with a drying oven set at 105°C. Crude ash content was measured using a dry ashing method at 550°C. Crude protein content was determined by using the micro-Kjeldahl method, which involved adding a protein decomposition promoter and sulfuric acid to the sample to facilitate protein breakdown, followed by quantification using a FOSS Kjeltec 8400 automatic protein analyzer. Crude fat content was analyzed by using the Soxhlet extraction method. Dietary fiber content was determined by using the enzymatic-gravimetric method.

Analysis of Amino Acid Composition

Amino acid composition was analyzed by using the ninhydrin reaction method according to the AOAC guidelines. For hydrolysis, 50 mg of each sample was placed in a bottle with 40 ml of 4N-hydrochloric acid under a nitrogen gas atmosphere to prevent oxidation and was heated at 110°C for 24 hours. Following hydrolysis, hydrochloric acid was evaporated under vacuum at 50°C, the residue was reconstituted with 50 ml of 0.2N sodium citrate buffer (pH 2.2), and the solution was then filtered through a 0.45 μm filter paper (Pall Life Sciences, California, USA). Finally, the filtered samples were analyzed using an amino acid analyzer (L-8900 High-Speed Amino Acid Analyzer, Hitachi, Tokyo, Japan).

Analysis of Fatty Acid Composition

Saturated and unsaturated fatty acids were analyzed following the fatty acid test methods outlined in the Food Codex and the extraction protocol developed by Folch *et al*. (1956). For lipid extraction, 50 g of each homogenized sample was extracted using 250 ml of chloroform:methanol (2:1) at 3,000 rpm in a homogenizer. After hydrolyzing the lipids, the fatty acids in the samples were analyzed by gas chromatography (GC model US/HP 6890, Agilent Technologies, Seoul, Korea), which used a silica capillary column (SP-2560, 100 m × 0.25 mm × 0.20 μm). The injection temperature was set at 225°C and 1 μl of each sample was injected for fatty acid analysis.

Analysis of Mineral Composition

Compositions of minerals and heavy metals were analyzed according to the regulations specified in the Food Codex. For analyzing trace elements and heavy metals, 50 mg of each oven-dried sample was subjected to preliminary ashing. Mineral analysis involved drying, carbonizing, and completely ashing the samples keeping the temperature between 450°C and 550°C. After adding hydrochloric acid, the solution was filtered through a glass fiber filter, and the content was measured using an inductively coupled plasma optical emission spectrometer (ICP-OES; Horiba, Kyoto, Japan). For heavy metal analysis, samples were ashed for over two hours in an electric furnace at 600°C. Subsequently, these were dissolved in 1:1 hydrochloric acid solution and allowed to stand for 18 hours. The dissolved samples

were filtered through Whatman No. 6 filter paper (Whatman International Co., Maidstone, UK). Next, the heavy metal content was analyzed using ICP-OES. The samples were analyzed using ICP at specific wavelengths for each element: lead (Pb) at 207 amu, cadmium (Cd) at 111 amu, and arsenic (As) at 75 amu.

Analysis of Harmful Composition

To ensure the safety of each sample, the presence of the hygiene indicator bacterium *Escherichia coli* O157:H7 was tested according to the Food Codex regulations. A sample of 25 g was taken, mixed with 225 ml of 0.1% sterile peptone water (BD, Sparks, MD, USA) and homogenized for 2 minutes using a homogenizer (Bag Mixer 400, Interscience, St. Nom, France). The prepared test solution was serially diluted by transferring 1 ml into 9 ml of sterile 0.1% peptone water, diluting it tenfold at each step. For the quantitative analysis of *E. coli*, 1 ml of the prepared test solution was inoculated onto two *E. coli*/coliform count Petrifilms (EC/CC, 3M) and incubated at 37°C for 48 hours. Colonies that formed a blue color with bubbles around them on the Petrifilm were identified as *E. coli* positive. Total aflatoxin and ochratoxin A were selected and identified as mycotoxins. Aflatoxin standards were prepared using a Mix Kit 4 solution (Supelco, Pennsylvania, USA), and ochratoxin A was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Toxin levels were measured using FLD detector (Nanospace Sl-2, Shiseido, Tokyo, Japan).

Statistical Analysis

One-way analysis of variance was conducted to assess the larval survival rate, weight, and development period across various food sources. All statistical analyses were performed using the statistical package SPSS PASW 22.0 for Windows (IBM, Chicago, USA).

Results and Discussion

Development of *P. brevitarsis* Larvae with Different Food Sources

The survival rates of *P. brevitarsis* larvae fed with OMCB or CB + OMCB were compared to those fed with FOS. This experiment was conducted until all the larvae formed cocoons. Therefore, the duration of this experiment was eight weeks for all groups. The group fed with FOS did not have any pupae until the sixth week and their survival rate was 100% (Fig. 1). Until the fifth week, before pupation occurred, the survival rates were 92.2–100% in the group fed with OMCB and 98.9–100% in the group fed with CB + OMCB. By the sixth week, pupation began in the experimental groups with survival rates of 73.3% in the OMCB group and 83.3% in the CB + OMCB group. Although the survival rates of *P. brevitarsis* larvae fed with OMCB and CB + OMCB were lower than those of the control group, all treatment groups presented survival rates of over 90% by the fifth week, indicating that stable rearing of *P. brevitarsis* larvae is possible.

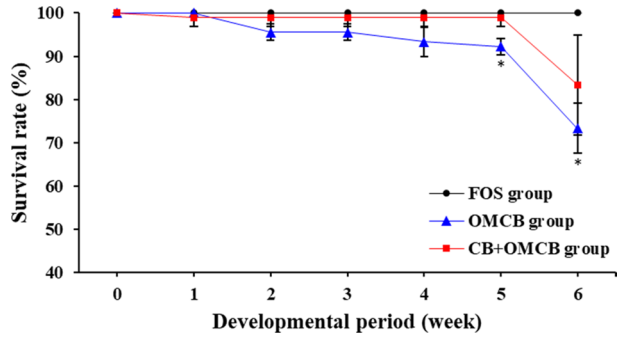

Fig. 1. Survival rate of Protaetia brevitarsis larvae fed fermented oak sawdust (FOS), fermented oyster mushroom cultivation by-products (OMCB) and coffee by-products (CB)
Note: FOS group, *P. brevitarsis* larvae fed with FOS; OMCB group, *P. brevitarsis* larvae fed fermented OMCB; CB + OMCB group, *P. brevitarsis* larvae fed fermented 50% of CB and 50% of OMCB. The values showed as means ± S.D. (n = 3). Differences were tested with one-way analysis of variance (ANOVA) followed by Tukey test. *, $p < 0.05$. All experimental groups were replicated thrice.

Compared to the control group fed with FOS, the OMCB and CB + OMCB groups were 1.7–3.7 and 1.5–4.2 folds higher by the sixth week, respectively. Although the control group fed with FOS reached a harvest weight of 2568.6 mg at the eighth week, the OMCB (2547.9 mg) and CB + OMCB (2700 mg) groups reached their harvest weights at the fifth week (Fig. 2). The addition of CB and OMCB is thought to have a direct effect on the body weight of *P. brevitarsis* larvae. These results indicate that when the harvest time for edible insects is considered to be at the third instar larval stage with a weight of 2500 mg, the larvae reach the harvest point by the sixth week, demonstrating the efficiency of the OMCB and CB + OMCB diets. Additionally, shortening the rearing period by two weeks can advance the harvest time of the larvae, which is expected to increase economic efficiency by reducing feeding costs for farming households during the shortened period.

Fig. 1 illustrates the results of feeding *P. brevitarsis* larvae

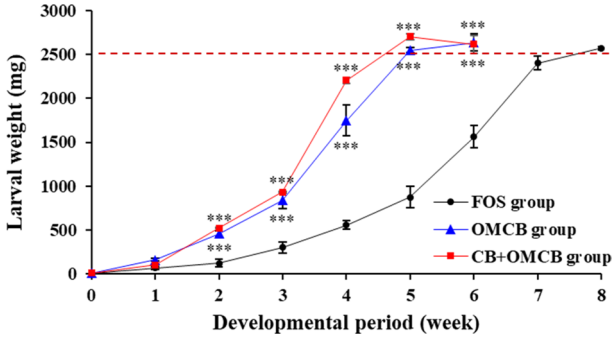

Fig. 2. Average larval weight of Protaetia brevitarsis larvae fed fermented oak sawdust (FOS), fermented oyster mushroom cultivation by-products (OMCB) and coffee by-products (CB)
Note: FOS group, *P. brevitarsis* larvae fed with FOS; OMCB group, *P. brevitarsis* larvae fed fermented OMCB; CB + OMCB group, *P. brevitarsis* larvae fed fermented 50% of CB and 50% of OMCB. The values showed as means ± S.D. (n = 3). Differences were tested with one-way analysis of variance (ANOVA) followed by Tukey test. ***, $p < 0.001$. All experimental groups were replicated thrice.

with coffee grounds and SMS deposits. The larval rearing period varied depending on diet. The shortest rearing period was observed in the group fed with CB + OMCB, which reached a harvest weight of 2500 mg in 32 days. The group fed with OMCB reached the same weight in 35 days, whereas the group fed with FOS required 53 days. Thus, feeding substrate CB + OMCB to *P. brevitarsis* larvae reduced the rearing period by approximately 40% compared to feeding with traditional FOS.

Comparative Analysis of the Nutritional Composition of Alternative Foods for *P. brevitarsis* Larvae

In the control group FOS and the experimental groups OMCB and CB + OMCB, we analyzed the nutritional components, including cell well constituents (e.g., neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, and lignin), proximate composition (e.g., moisture and crude protein, fat, and ash), caloric content, and 17 types of amino acids.

Table 1 presents the findings of the proximate composition analyses of the different types of alternative feeds. Crude protein content was 2.2 and 3.2 folds higher in OMCB and CB + OMCB groups, respectively, than in the control group. OMCB and CB + OMCB had significantly higher protein contents than the control group. The high protein content of food sources is thought to be related to the protein content of the *P. brevitarsis* larvae, suggesting the necessity for further research on this relationship. Cellulose content, which served as a nutrient source for the larvae, was 1.3 folds higher in the CB + OMCB group than in both the control and OMCB groups. The effect of food source variation on the nutritional composition of *P. brevitarsis* larvae surpasses that of conventional livestock, primarily because of their concise digestive system (Yoon *et al*., 2020). Furthermore, it has been postulated that similar to conventional livestock, the nutritional composition of *P. brevitarsis* larvae can be influenced by food sources, leading to differences in common ingredients

Table 1. Composition of the nutrients in food sources fermented oak sawdust (FOS), fermented oyster mushroom cultivation by-products (OMCB) and coffee by-products (CB)

Components		Standard feed	Alternative feed	
		FOS	OMCB	CB + OMCB
General composition	Calorie (cal/g)	2766	3226	4092
	Moisture (%)	42.0 ± 2.8	26.9 ± 1.5 *	14.5 ± 0.0 *
	Crude protein (%)	3.9 ± 0.2	8.5 ± 0.1 **	12.5 ± 0.3 ***
	Crude fat (%)	0.6 ± 0.3	0.6 ± 0.2	0.8 ± 0.3
	Crude ash (%)	1.5 ± 0.1	3.7 ± 0.2 *	2.8 ± 0.1 **
	Crude fiber (%)	28.9 ± 1.6	33.9 ± 1.6	33.6 ± 0.1
Cell membrane components	Cellulose (%)	22.7 ± 1.2	23.5 ± 1.3	29.9 ± 0.2
	Lignin (%)	11.8 ± 0.1	21.1 ± 0.5 *	21.4 ± 0.4 *
	NDF (%)	47.2 ± 2.2	56.0 ± 2.2 *	66.4 ± 0.3 *
	ADF (%)	34.5 ± 1.1	44.6 ± 1.8	51.3 ± 0.7

Note: NDF, neutral detergent fiber; ADF, acid detergent fiber. The values showed as mean ± S.D. (n = 2). Differences were tested with one-way analysis of variance (ANOVA) followed by Tukey test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. CB + OMCB, fermented 50% of coffee by-products and 50% of oyster mushroom cultivation by-products.

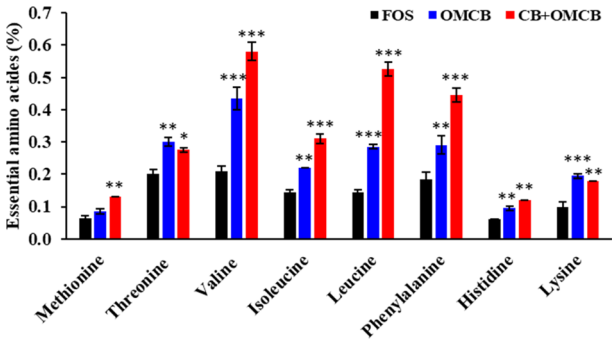

Fig. 3. Essential amino acid content of food sources fermented oak sawdust (FOS), fermented oyster mushroom cultivation by-products (OMCB) and coffee by-products (CB)
Note: CB +OMCB, fermented 50% of CB and 50% of OMCB. The values showed as means ± S.D. (n = 2). Differences were tested with one-way analysis of variance (ANOVA) followed by Tukey test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. All experimental groups were replicated thrice.

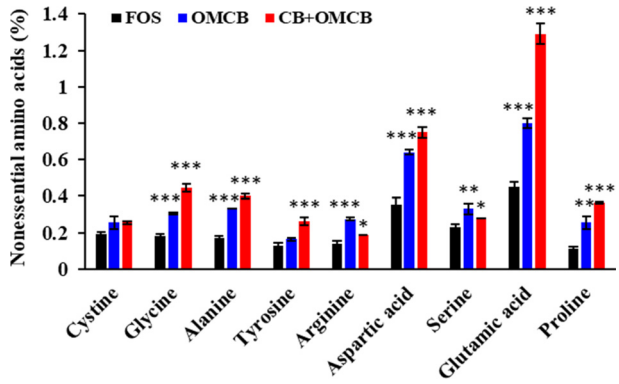

Fig. 4. Nonessential amino acid content of food sources fermented oak sawdust (FOS), fermented oyster mushroom cultivation by-products (OMCB) and coffee by-products (CB)
Note: CB + OMCB, fermented 50% of CB and 50% of OMCB. The values showed as means ± S.D. (n = 2). Differences were tested with one-way analysis of variance (ANOVA) followed by Tukey test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. All experimental groups were replicated thrice.

(Choi *et al*., 2019). Therefore, considering the high protein and dietary fiber content, it is beneficial to provide CB + OMCB feed when utilizing *P. brevitarsis* larvae as edible insects.

Alternative food sources were analyzed for their amino acid compositions, as depicted in Figs. 3 and 4. Valine, an essential branched-chain amino acid, was found to be 2 and 3 folds higher in OMCB and CB + OMCB groups, respectively, than in the control group. Similarly, leucine content showed a threefold and fivefold increase in the OMCB and CB + OMCB groups, respectively, relative to the control group. Valine, isoleucine, and leucine—which are primarily found in meat, fish, dairy, eggs, and nuts—are essential amino acids that play crucial roles in muscle tissue growth, recovery, and blood sugar regulation (Anthony *et al*., 2001; Atherton *et al*., 2010; Yu *et al*., 2021).

Among the non-essential amino acids, glutamic acid, which is known for its umami taste, was 2 and 3.3 folds higher in OMCB and CB + OMCB groups, respectively, than in the control group. Proline, which is known to aid collagen synthesis and cartilage formation (Liang *et al*., 2013), showed approximately 3 and 4 folds higher levels in OMCB and CB + OMCB groups, respectively, than in the control group. Aspartic acid content was approximately 1.5 and 1.8 folds elevated in OMCB and CB + OMCB groups, respectively, than that in the control group. Compared with the control group, food sources OMCB and CB + OMCB exhibited higher concentrations of aspartic acid, glutamic acid, and tyrosine, which are known to function as neurotransmitters (Holeček, 2023; Jongkees *et al*., 2015; Niciu *et al*., 2012). Compared with the control, OMCB showed higher concentrations of arginine, which is crucial for improving blood circulation and enhancing immune function (Mori *et al*., 1998). Additwionally, proline, which is essential for collagen synthesis and aids in cellular defense against oxidative stress (Liang *et al*., 2013), was present at higher concentrations in both OMCB and CB + OMCB groups. Therefore, food sources containing CB and OMCB are regarded as nutritionally superior to *P. brevitarsis* larvae. Owing to the well-balanced distribution of various minerals, CB + OMCB is considered to have significant potential as a food source for *P. brevitarsis* larvae.

Hazardous Material Analysis

In the hazardous material analysis, the heavy metals—Pb, As, Cd, mycotoxin, total aflatoxin, and ochratoxin A—were checked, and the results are summarized in Tables 2 and 3. In the case of Pb, the allowable standards for animal feed and fiber feedstuff were 10 mg/kg. Both As and Cd met the standards, and Pb was not detected in the control group. Cd and As were not detected in any food sources. However, mycotoxin, total aflatoxin, and ochratoxin A were not detected in any food source. These results indicate that this feed is safe for use as a food source for *P. brevitarsis* larvae. The absence of these contaminants ensures that the feed does not pose any risk of toxicity, making it a reliable and safe option for promoting healthy growth and development of *P. brevitarsis* larvae.

Table 2. Heavy metals in food sources fermented oak sawdust (FOS), fermented oyster mushroom cultivation by-products (OMCB) and coffee by-products (CB)

Hazardous substance	Content	Standard feed	Alternative feed	
		FOS	OMCB	CB + OMCB
Heavy metals (mg/kg)	Lead (Pb)	0.00	1.1 ± 0.2	0.8 ± 0.1
	Cadmium (Cd)	0.00	0.00	0.00
	Arsenic (As)	0.00	0.00	0.00

Note: The values showed as means ± S.D. (n = 2). CB + OMCB, fermented 50% of coffee by-products and 50% of oyster mushroom cultivation by-products.

Table 3. Pathogenic microbes in food sources fermented oak sawdust (FOS), fermented oyster mushroom cultivation by-products (OMCB) and coffee by-products (CB)

Hazardous substance	Content	Standard feed	Alternative feed	
		FOS	OMCB	CB + OMCB
Pathogenic microbes	Ochratoxin A	N.D.†	N.D.†	N.D.†
	Total aflatoxins (Aflatoxin B1, B2, G1, G2)	N.D.†	N.D.†	N.D.†

Note: N.D.†, Not detected. CB + OMCB, fermented 50% of coffee by-products and 50% of oyster mushroom cultivation by-products.

Conclusion

According to the abovementioned results, the following was noticed. Crude protein content in OMCB and CB + OMCB was 2.2–3.2 folds higher than that in FOS. Crude fiber content was 33.9% in OMCB and 33.6% in CB + OMCB, which was 1.16–1.17% higher than that in FOS (28.9%), and crude fiber content was 29.9% in OMCB, which was 1.32 folds higher than that in FOS (22.7%). Crude ash content was 1.9 folds higher. OMCB and CB + OMCB contain high amounts of nutrients that cannot be synthesized in the body, such as unsaturated fatty acids, and their safety was verified through the analysis of harmful substances, such as heavy metals and mycotoxins. Additionally, food sources containing coffee grounds and mushroom cultivation by-products are considered to have high potential as food source for *P. brevitarsis* larvae because of their high protein and crude fiber content and rich mineral composition, which are beneficial for larval growth and development.

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