

Nutritional Value, Microbiological Safety, and Mycotoxin Risk of Black Soldier Fly Larvae: Implications for Dog Nutrition

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Abstract: This study comprehensively evaluates the crude chemical composition, microbiological dynamics, and mycotoxin contamination in *Hermetia illucens* larvae (Black Soldier Fly Larvae, BSFL), aiming to assess their suitability as a sustainable protein source. Proximate analysis revealed a high protein content (43.22%), along with significant fat levels (19.99%) and moderate fiber content (12.05%), predominantly chitin. Mycotoxin analysis indicated safe levels of aflatoxin B1 (1.29 µg/kg) and deoxynivalenol (6.0 µg/kg), with undetectable levels of ochratoxin A, ensuring compliance with feed safety standards. Microbiological assessments across developmental stages identified a progressive increase in microbial load, in adults. The predominant microbial species included *Enterococcus* spp., *Klebsiella aerogenes*, and *Escherichia coli*. Thermal treatment via microwave drying significantly reduced microbial contamination, although *Enterococcus* spp. remained detectable post-treatment. These findings highlight BSFL's potential as a nutritionally valuable ingredient in animal feed, particularly due to their high protein and fat content. However, further refinement of microbial decontamination strategies is necessary to enhance safety, ensuring their optimal use in food and feed applications.

Keywords: insect, dog, nutrition, analysis

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1. Introduction

The global demand for sustainable and high-quality protein sources is increasing, driven by population growth, environmental concerns, and the need to reduce reliance on conventional livestock production. Insects, particularly Black Soldier Fly Larvae (BSFL), have emerged as a promising alternative protein source for both animal feed and human consumption due to their high nutritional value and low environmental footprint [1,2]. BSFL are rich in protein, essential fatty acids, and micronutrients, making them an attractive candidate for various applications [3], including pet food [4]. In particular, insect-based ingredients are gaining attention in the pet food industry, where dog nutrition is being re-examined through the lens of sustainability and alternative protein sources [5]. The Black Soldier Fly (*Hermetia illucens*) is a non-pest species of *Diptera* native to tropical and temperate regions worldwide [6]. It has gained significant attention for its unique ability to convert organic waste into valuable biomass, reducing environmental waste while producing nutrient-dense larvae [7]. The life cycle of *H. illucens* is divided into several key stages: egg, larva, pupa, and adult, with each stage playing a vital role in its biological and ecological success [8].

The use of Black Soldier Fly Larvae in dog nutrition is increasingly being explored due to the high protein content and essential fatty acids comparable to traditional protein

sources like chicken or fish. BSFL offers additional benefits, including being rich in medium-chain fatty acids, such as lauric acid, which can promote immune function and improve skin and coat health in dogs [9]. As dogs require high-quality protein for muscle maintenance and overall health, BSFL represents a nutritionally balanced and environmentally sustainable ingredient that could reduce the ecological footprint of pet food production. Moreover, BSFLs are hypoallergenic, making them a potential solution for dogs with food sensitivities to common proteins like beef or poultry [10]. However, for BSFL to be integrated into dog food, microbiological safety is a critical concern. Insects, like BSFL, naturally harbor microbes, which can include both beneficial bacteria and harmful pathogens such as *Salmonella* spp. [11] and *Escherichia coli* spp. [12]. Ensuring that BSFL-derived products are microbiologically safe is essential for preventing health risks in pets [13]. Various rearing conditions and substrates, like wheat bran, can influence microbial loads [14]. By evaluating microbial populations at different developmental stages, from larvae to adults, and using thermal treatments to reduce microbial contamination in BSFL powder, this study aims to ensure that BSFL meets the stringent safety standards required for dog food ingredients. Additionally, the presence of mycotoxins, which can pose a risk to pet health, is assessed to ensure compliance with pet food safety guidelines.

This study focuses on BSFL reared on wheat bran, exploring their chemical composition, microbial dynamics, and mycotoxin contamination to assess their suitability as a safe and nutritious ingredient for dog food. By integrating the nutritional benefits and addressing safety concerns, this research supports the potential of BSFL as a sustainable, eco-friendly solution for the pet food industry.

2. Materials and Methods

Black Soldier Fly Larvae (BSFL), adults, pupae and eggs were purchased from an industrial facility located in Brasov County, Romania. The larvae were delivered dried and were subsequently ground to a fine powder in preparation for further analyses. **Chemical Composition Analysis:** The powder was analyzed for crude protein, fat, moisture, fiber, NFE (nitrogen-free extract) and ash content, following standard procedures. **Mycotoxin Testing:** The powder was also assessed for the presence of mycotoxins, including aflatoxins and ochratoxins, to ensure compliance with safety standards for pet food ingredients. **Nutritional Value:** samples were homogenized before analysis to ensure consistency. The gross chemical composition, including moisture, crude protein, crude fiber, crude fat, NFE (nitrogen-free extract) and ash content, was determined following the procedures outlined in the AOAC official methods. To analyze the chemical composition of Black Soldier Fly larvae, specific devices and methods are employed. Dry matter content is determined using a drying oven, such as the Memmert Universal Oven UF. Crude protein is measured via the Kjeldahl method, utilizing a semi-automatic device like the Kjeltac 8400 Analyzer Unit. The ether extract is obtained through the Soxhlet method, using a Soxhlet apparatus. Crude ash was quantified using a muffle furnace in accordance with standard incineration methods. The Nitrogen-Free Extract (NFE) was calculated by subtracting the sum of the crude protein, ash, ether extract, and crude fiber from 100% of the dry matter, as per standard proximate analysis methods. This calculation provides an estimate of the carbohydrate content.

Microbiological Analysis: To evaluate the microbiological safety and monitor microbial dynamics throughout the developmental stages, a total of 8 samples were examined. One sample was taken from each key life stage of the larvae, including adults, live eggs, first and second-instar larvae, third and fourth-instar larvae, pupae, and microwave-dried larvae. Each sample was analyzed for microbial load and species composition to determine the progression of microbial communities during larval development and post-processing treatment. ***Eggs:** Purchased immediately after oviposition. ***First Instar:** Purchased 3 days post-hatching. ***Second Instar:** Purchased 8 days post-hatching. ***Third Instar:** Purchased 12 days post-hatching. ***Fourth Instar:** Purchased 18 days post-hatching. ***Fifth Instar:** Purchased on the day of slaughtering (22 days post-hatching). ***Pupae:** Purchased at 24 days post-hatching. ***Adults:** Purchased immediately upon emergence. The samples were analyzed using quantitative and qualitative methods. **Quantitative analysis:** The serial dilution method was employed to estimate the number of microorganisms. Initially, 0.5 g of the sample was diluted in 4.5 ml of sterile saline to obtain a 1:10 dilution. Serial dilutions (up to 10^{-5}) were prepared similarly. From each dilution, 0.5 ml were plated on nutrient agar (Merck, Darmstadt, Germany) and incubated at 37°C for 24 hours. The number of colonies formed was calculated using the following formula: $TGN = \text{number of colonies} \times \text{dilution factor} \times 1/\text{volume plated}$. **Qualitative analysis:** For bacterial identification, samples from the final dilutions were inoculated on MacConkey agar (BioMaxima S.A., Lublin, Poland) and UriSelect medium (Bio-Rad Laboratories Inc., Hercules, CA, USA) URI chromogenic medium. Colonies that

could not be identified based on morphological or cultural characteristics were further analyzed using the Vitek® 2 Compact device (BioMerieux, Marcy l'Etoile, France), which determines 64 biochemical characteristics of bacteria. Mycotoxin Analysis: for the mycotoxicological examination, the test of RIDASCREEN®FAST Aflatoxin, RIDASCREEN®FAST Ochratoxin A and RIDASCREEN®FAST Deoxynivalenol were used, competitive enzyme immunoassay tests for the quantitative determination of total aflatoxin, ochratoxin A and deoxynivalenol in cereals and food. The basis of the test is the antigen-antibody reaction. The measurement was performed photometrically at 450 nm using Awareness Technology Model 4300 Chromate Microplate Reader.

3. Results

The chemical composition analysis of *Hermetia illucens* larvae was conducted to evaluate their nutritional potential, focusing on key parameters such as water content, dry matter, crude protein, crude fat, NFE (nitrogen-free extract), and ash content. The laboratory analysis revealed a water content of 9,70%, contributing to a dry matter (DM) content of 90,30%. This dry matter was composed predominantly of organic matter (82,76% of the sample, or 91,65% of DM), while ash content, representing mineral salts, accounted for 7,54% of the sample (8,35% of DM). Protein analysis demonstrated a substantial nitrogenous content, with crude protein making up 43,22% of the sample, equivalent to 47,86% of DM. Furthermore, the fat content measured 19,99% of the total sample (22,14% of DM), underscoring the larvae's suitability as a source of essential lipids. The fiber content of 12,05% (13,34% of dry matter), is substantial for promoting gastro-intestinal health in dogs. A significant portion of this fiber is represented by chitin, a natural polysaccharide found in the exoskeletons of insects. Chitin is known for its prebiotic properties, contributing to the development of beneficial gut bacteria, and promoting overall digestive health in dogs [15]. However, chitin is not as easily digestible as other fibers, meaning that while it has nutritional benefits, it must be complemented with other easily digestible ingredients to ensure a well-rounded diet. The nitrogen-free extract (NFE), at 7,50%, consists mainly of digestible carbohydrates, providing an important source of energy. Despite its nutritional value, the moderate levels of NFE and fiber indicate that this insect powder should be included as a supplementary ingredient in dog food formulations, rather than being used as a sole dietary component. *Hermetia illucens* larvae reared on wheat bran exhibit a high protein and fat content, along with moderate levels of ash, indicating a rich mineral composition, as well as moderate amounts of fiber and nitrogen-free extract (carbohydrates). While these nutritional properties make them a promising ingredient for dog food, particularly for fulfilling protein and essential fatty acid requirements, it is recommended that the larvae powder be used as a supplementary ingredient rather than a singular, complete meal for dogs.

Table 1. Values obtained from the performed determinations (chemical composition)

GROSS CHEMICAL COMPOSITION	% of sample	% of DM
Water	9,70	-
Dry matter	90,30	100
Ash	7,54	8,35
Total organic matter	82,76	91,65
Protein	43,22	47,86
Fat	19,99	22,14
Fibres	12,05	13,34
NFE	7,50	8,31

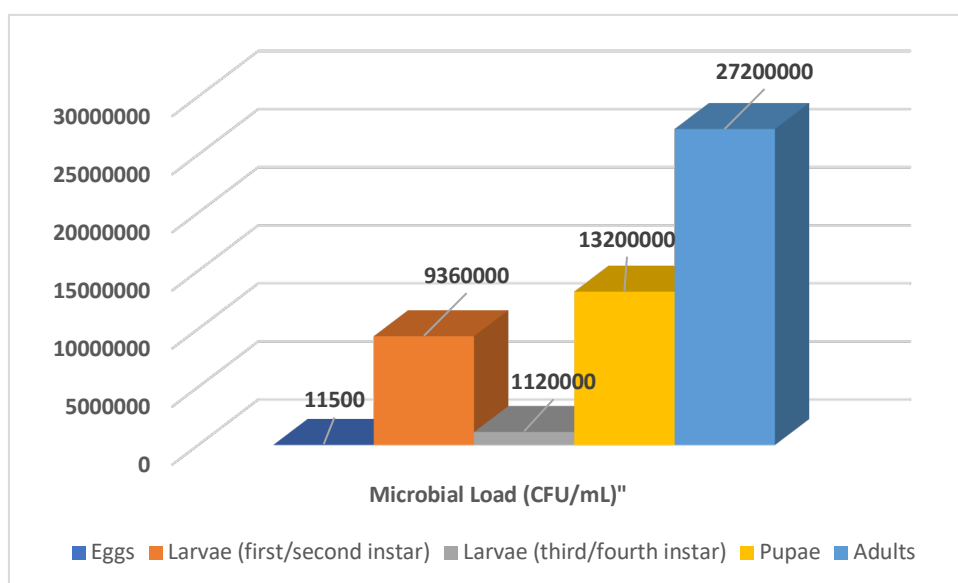
NFE- nitrogen-free extract; DM-dry matter

The detected concentrations of various mycotoxins in the analyzed samples are presented in Table 2. Aflatoxin B1 was measured at 1.29 µg/kg, while Deoxynivalenol was found at 6.0 µg/kg. Ochratoxin A levels were undetectable in the samples.

Table 2. Mycotoxin Levels in BSF Larvae

Mycotoxin	Detected Level ($\mu\text{g}/\text{kg}$)
Aflatoxin B1	1.29
Deoxynivalenol	6.0
Ochratoxin A	0.0

The microbial load and species diversity across different life stages and processing conditions of wheat bran-fed insects were assessed through quantitative and qualitative tests (Table 3). The highest microbial load was observed in the adults, with a concentration of 2.72×10^7 CFU/mL. The alive larvae (first/second instar) showed a microbial load of 9.36×10^6 CFU/mL while for the third/fourth instar, we managed to detect 1.12×10^6 CFU/mL. For the alive eggs, microbial counts were found at 9.0×10^3 CFU/mL and 1.0×10^4 CFU/mL. Alive pupae expressed a microbial load of 1.32×10^7 CFU/mL. Microwave-dried larvae showed lower microbial levels, with 2.1×10^5 CFU/mL and 1.6×10^4 CFU/mL (Table 3). The microbial load in black soldier flies increases progressively from eggs to adults, with the highest concentration observed in the adult stage (2.72×10^7 CFU/mL), compared to lower levels in eggs (9.0×10^3 to 1.0×10^4 CFU/mL) and other developmental stages (Figure 1).

**Figure 1.** Quantitative test - Microbiology essay

A qualitative microbiological analysis was conducted to evaluate the microbial presence at various developmental stages of *Hermetia illucens* (Black Soldier Fly). The results showed that in adults, the microbial species included *Enterococcus* spp., *Proteus mirabilis*, *Myroides* spp., and *Providencia rettgeri* was detected. For the eggs, *Enterococcus* spp. was the only isolated genus. In the first and second instar larvae cultivation showed the presence of *Klebsiella aerogenes*, *Escherichia coli*, *Myroides* spp., *Enterococcus* spp., *Klebsiella pneumoniae* ssp. *pneumoniae*. In the third and fourth instar larvae, *Escherichia. coli*, *Enterococcus* spp., *Klebsiella aerogenes*, *Myroides* spp. and members of the *Enterobacter cloacae* complex were present. The analysis of the pupae indicated the presence of *Enterococcus* spp. and *Klebsiella aerogenes*.

Furthermore, in microwave-dried larvae, only *Enterococcus* spp. showed any growth. These results demonstrate that the microbial diversity changes across developmental stages of *Hermetia illucens*. In particular, larvae exhibit a more diverse microbiota compared to pupae and adults, with a predominance of *Enterococcus* spp. and *Klebsiella* spp.. Thermal treatment, such as microwave drying, reduces bacterial contamination significantly but does not eliminate it, as evidenced by the persistence of *Enterococcus* spp. in the dried larvae.

Table 3. Results of qualitative and quantitative examination of Black Soldier Fly larvae /adults/pupae/ eggs fed

Product name	Adults	Alive eggs	Alive larvae – First/Second instar	Alive larvae – Third/Fourth instar	Alive pupae	Microwave-dried wheat bran-fed larvae
Quantitative test (TNG-CFU/mL)	10 ⁻¹	-	-	-	-	-
	10 ⁻²	-	9000	-	-	-
	10 ⁻³	-	10000	-	11200000	210000
	10 ⁻⁴	-	-	-	132120000	160000
	10 ⁻⁵	272800000	-	93600000	-	-
Quality review	URI	10 ⁻¹ Enterococcus spp., Proteus mirabilis, Myroides spp.	Enterococcus spp.	Klebsiella aerogenes, E. coli, Myroides spp., Enterococcus spp.	E. coli, Enterococcus spp., Klebsiella aerogenes, Myroides spp.	Enterococcus spp., Klebsiella aerogenes
	MAC	10 ⁻¹ 10 ⁻² 10 ⁻³	Providencia rettgeri	0	E. coli, Klebsiella pneumoniae ssp. Pneumoniae	E. coli, Enterobacter cloacae complex
						0

4. Discussion

The gross chemical composition of *Hermetia illucens* larvae in our study aligns with previously reported findings, particularly for dry matter content, supporting the consistency of the larvae's moisture retention and nutrient concentration [3]. Our protein analysis showed levels comparable to those cited in the literature, reinforcing the larvae's role as a protein-rich resource for both animal and human nutrition [16]. The stable protein content, even with minor substrate variations, suggests that wheat bran is a viable alternative for maintaining nutritional value in pet diets.

In addition to protein, the larvae powder is rich in lipids and minerals, while providing moderate amounts of fiber and carbohydrates. Although the measured fat and ash content were slightly below typical ranges [3, 20], they still confirm the larvae's potential as a significant lipid source with a valuable mineral profile, particularly for calcium and phosphorus, which are essential for canine health [17]. Moreover, the presence of chitin, known for its prebiotic properties, highlights the potential gut health benefits of incorporating *Hermetia illucens* larvae into dog diets [18].

Moreover, *Hermetia illucens* larvae powder is already used as an ingredient in several commercial recipes for extruded dry dog food due to its balanced nutrient profile and environmental sustainability [19]. Beyond its industrial applications, the powder could also serve as an ingredient in homemade dog feed or treats, offering pet owners a sustainable and nutrient-dense alternative [20]. However, despite the impressive nutritional qualities of *Hermetia illucens* larvae powder, it is not suited to be the sole ingredient for balanced canine nutrition. While it provides high levels of protein, fat, and minerals, a complete and balanced diet requires a more diverse range of nutrients that cannot be met by insect powder alone. Therefore, this ingredient should be used as part of a broader nutritional strategy to meet all of a dog's dietary needs [21]. Our study aimed to assess the quality of insect meal, taking *Hermetia illucens* larvae in their dried form, grinding them ourselves, and evaluating the nutritional properties of the resulting powder. Specifically, we

sought to determine whether the substrate used to feed the larvae influences the nutritional composition of the insect powder and assess its potential for inclusion in dog nutrition. Through this analysis, we have contributed to understanding the versatility and limitations of *Hermetia illucens* powder as a functional ingredient in canine diets.

The analysis of mycotoxins in *Hermetia illucens* (Black Soldier Fly) larvae revealed relatively low contamination levels compared to the maximum allowable limits set by the European Union for dogs [22]. Specifically, Aflatoxin B1 was measured at 1.29 µg/kg, Deoxynivalenol (DON) at 6.0 µg/kg, while Ochratoxin A was below detectable levels. These findings are significant as they suggest that BSF larvae raised on wheat bran present a low risk of acute mycotoxin toxicity for animals, including dogs. When compared to the maximum admitted levels, which are 20 µg/kg for Aflatoxin B1(C, 2006), 5,000 µg/kg for Deoxynivalenol [22], and 10 µg/kg for Ochratoxin A [22], the detected concentrations in our study are far below the thresholds. This suggests that feeding BSF larvae to dogs would not likely lead to acute mycotoxicosis, a condition that typically occurs following the ingestion of high mycotoxin concentrations. However, while the acute risk is minimal, the potential for chronic exposure should not be ignored. Prolonged consumption of feed containing low levels of mycotoxins could predispose dogs to long-term health issues, such as liver disease or cancer, particularly in the case of Aflatoxin B1 [23]. Chronic aflatoxin exposure has been linked to hepatotoxicity and hepatocellular carcinoma in various animal species, including dogs, even at subclinical levels. This raises concerns about the potential cumulative effects of long-term, low-level mycotoxin ingestion in pet diets [24]. To mitigate chronic exposure to mycotoxins in animal diets, particularly for dogs, it is crucial to implement effective monitoring systems and establish clear thresholds for mycotoxin levels in feed ingredients such as Black Soldier Fly larvae. While commercial dry dog food typically shows mycotoxin contamination levels below regulatory limits, the risk of chronic exposure from consistent, low-level intake remains a concern. Dogs may be exposed daily to small quantities of mycotoxins, which, over time, could pose health risks. Regular testing of both the larvae and their substrates, as well as periodic monitoring of mycotoxins in dog feed, is essential to ensure safety [25]. Further processing methods, including thermal treatments and the use of detoxifying additives, can help significantly reduce mycotoxin concentrations. Additionally, high-quality substrate management is vital, as it directly affects the nutritional value and safety of the larvae as a feed source. Prioritizing these strategies will enhance the health and well-being of pets and livestock, minimizing the long-term risks associated with mycotoxin exposure [26]. Despite the low levels of mycotoxins detected in BSF larvae, the literature suggests that BSF larvae have a unique ability to degrade or tolerate certain contaminants, including mycotoxins. Previous studies have highlighted that Black Soldier Fly larvae can degrade aflatoxins and other harmful compounds during digestion, reducing the risk of contamination in the final product [27]. However, the efficacy of this bioconversion varies depending on the type of mycotoxin and the concentration present in the feed substrate [28]. For instance, some research has shown that BSF larvae can significantly reduce Aflatoxin B1 levels in contaminated substrates, although complete degradation may not always occur [29]. This ability to partially detoxify their feed could explain the low mycotoxin concentrations observed in our study, despite the presence of wheat bran, a substrate that can be prone to fungal contamination. Our findings align with this body of research, as the larvae's low mycotoxin levels indicate that wheat bran is a suitable substrate for BSF rearing without introducing significant risks of contamination. Nevertheless, further research is required to better understand the long-term effects of chronic exposure to low mycotoxin levels in both BSF larvae and the animals consuming them. For now, the results are promising in terms of the safety and sustainability of using BSF larvae as a protein source for dog nutrition, particularly given their low mycotoxin content and the larvae's inherent detoxification capabilities.

Our study explored the microbial load and species diversity across different developmental stages of *Hermetia illucens* (Black Soldier Fly) fed on wheat bran, without dissecting the larvae, pupae, or adult gut. Instead, we aimed to observe the bacterial dynamics throughout their development in a commonly used substrate, assessing microbial presence on the external body surface and internal gut as total. The results highlight significant changes in both microbial load and bacterial species composition as the larvae progressed through their life cycle, as well as the effect of thermal processing (microwave drying) on bacterial contamination. Quantitatively, the microbial load increased progressively from eggs to adults, with the highest load detected in the adults (2.72×10^7 CFU/mL at a dilution of 10^{-5}), while the lowest levels were recorded in the eggs (9.0×10^3 to 1.0×10^4 CFU/mL). Notably, the microbial load in the larvae also varied with developmental stage; first/second instar larvae exhibited a load of 9.36×10^6 CFU/mL, while the third/fourth instar larvae

showed a reduced load of 1.12×10^6 CFU/mL. These fluctuations in microbial load are consistent with findings in the literature [7], which emphasize that as insects grow, they change the microbiota due to shifts in their diet, metabolic activity, and immune system responses [30]. Qualitatively, our analysis revealed diverse microbial communities at different life stages. For example, first/second instar larvae hosted a more varied microbiota, including *Klebsiella aerogenes*, *Escherichia coli*, *Myroides* spp., and *Enterococcus* spp., while third/fourth instar larvae displayed similar species with the addition of members from the *Enterobacter cloacae* complex. This aligns with previous research [31] indicating that BSF larvae possess a dynamic microbiota, which can help degrade organic matter and enhance nutrient recycling. The decrease in microbial diversity observed as the larvae progressed to the pupal and adult stages, particularly the predominance of *Enterococcus* spp. and *Klebsiella* spp., has also been reported in other studies [32], suggesting that microbial diversity tends to narrow as insects undergo metamorphosis. The results highlight significant changes in both microbial load and bacterial species composition as the larvae progressed through their life cycle, as well as the effect of thermal processing (microwave drying) on bacterial contamination. This variation in microbial load across different developmental stages is crucial for optimizing thermal treatment methods, as certain life stages may require more stringent processing to ensure microbial safety. Practical solutions could involve tailoring the duration and intensity of heat treatment based on the larvae's developmental stage. For instance, larvae in later stages, which may harbour more resilient bacterial species, could benefit from longer microwave drying times or higher temperatures to ensure a more comprehensive microbial reduction [33]. Furthermore, our study aimed to assess whether thermal treatment via microwave drying could effectively reduce bacterial contamination in BSF byproducts to make them safer for consumption, particularly in animal feed. The results indicate that while microwave drying significantly reduced the microbial load—showing 2.1×10^5 CFU/mL and 1.6×10^4 CFU/—it did not eliminate microbial presence. *Enterococcus* spp. was still detected in dried larvae, a finding that aligns with the notion that while heat treatment is effective [34], complete sterility is difficult to achieve. These results are critical when considering the potential use of BSF larvae as a feed ingredient for dogs. Although thermal processing substantially reduces microbial contamination, the persistence of *Enterococcus* spp. is noteworthy. In the context of canine nutrition, this species, while commonly present in the environment, could pose a risk to immunocompromised dogs [34,35]. Chronic exposure to low levels of potentially pathogenic bacteria could increase the likelihood of infection or contribute to the development of other conditions in susceptible animals [36]. To mitigate this risk, future studies could explore combining microwave drying with other sterilization techniques, such as pressure-based methods (e.g., high-pressure processing), which have been shown to inactivate heat-resistant bacterial species. [37]

In comparison to findings in the literature [38], our study supports the general trend that microbial load and diversity fluctuate across the life stages of BSF, influenced by both the insect's developmental biology and the substrate composition. Additionally, the literature suggests that BSF larvae can reduce certain pathogens in their environment due to their microbial communities [39], yet some bacteria, such as *Enterococcus* spp., are resilient and can persist despite thermal processing. This highlights the practical importance of refining thermal treatment methods, such as adjusting drying times or temperatures, to target resilient bacteria more effectively. Solutions such as incorporating temperature gradients—where the drying process begins at lower temperatures to avoid nutritional degradation and gradually increases to ensure bacterial inactivation—could optimize both safety and quality. Additionally, the use of microbial inhibitors (e.g., organic acids) during the processing phase could further suppress bacterial survival while maintaining the larvae's nutritional integrity.

Therefore, our findings contribute to the ongoing understanding of microbial dynamics in BSF and underscore the need for further investigation into optimizing processing methods to ensure microbial safety without compromising the larvae's nutritional integrity. These insights could inform future processing standards, where variations in microbial load across different life stages are accounted for. Establishing specific protocols for each larval stage could lead to more precise drying times and temperatures, minimizing nutrient loss while maximizing microbial safety. A combination of tailored heat treatment, surface cleaning, and supplementary sterilization methods could be integrated into industrial processes to achieve the highest safety standards for BSF-derived products.

5. Conclusions

Our study highlights the nutritional viability of *Hermetia illucens* larvae as a protein-rich, sustainable ingredient for dog nutrition. The larvae's gross composition, particularly their high protein content, aligns well with the dietary needs of most animals. Despite slight deviations in fat and ash content compared to previous research, the larvae maintain a robust nutritional profile suitable for pet diets, supporting both energy needs and bone health. The low levels of mycotoxins detected, far below the EU-admitted thresholds, indicate minimal risk for acute toxicity in dogs. However, the potential long-term effects of low-level Aflatoxin B1 exposure warrant further investigation, particularly given its link to chronic liver disease. Additionally, our microbial analysis underscores the need for rigorous safety protocols in processing larvae for dog food, as *Enterococcus* spp. can pose a risk to immunocompromised individuals.

Black soldier fly larvae reared on wheat bran demonstrate great potential as a sustainable, nutrient-dense ingredient for dog food and animal feedings in general, offering high protein content with manageable mycotoxin contamination. While thermal processing effectively reduces bacterial load, optimizing this process for microbial safety remains crucial, especially for sensitive pets. However, while *Hermetia illucens* larvae powder has many desirable nutritional qualities, it cannot serve as a sole ingredient for a balanced canine diet. A complete and balanced diet for dogs requires a broader range of nutrients that cannot be provided by insect powder alone. Therefore, its role should be considered as a complementary ingredient within a more comprehensive dietary framework. Overall, our findings support the further investigation of *Hermetia illucens* larvae as a cost-effective, eco-friendly alternative protein source for not only pet nutrition but also for various animal feed industries. Its excellent nutritional profile, combined with its sustainable production, offers a promising solution for advancing animal feed formulations without compromising nutritional quality or safety across different species."

Supplementary Materials: Not applicable.

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