

Frass from black soldier fly larvae as an aquafeed ingredient: Nutritional value and potential health benefits

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Abstract: Insects have gained global research interest as potential protein sources, increasing insect production to meet the rising demand for protein. The insect meal production produces a considerable amount of frass, which consists of waste produced by larvae, leftover food, and exoskeleton remnants. Two separate trials were conducted to explore the nutritional value of frass generated from black soldier fly larvae, *Hermetia illucens* fed dried distillers' grains for channel catfish, *Ictalurus punctatus*, and hybrid tilapia, Nile x Mozambique (*Oreochromis niloticus* x *O. mossambicus*). Our study aimed to evaluate the impact of adding various levels of frass to fish diets on overall performance and health. Five isocaloric practical-type diets containing frass at 0% to 30% were formulated. Dietary frass served as a partial replacement for a combination of soybean meal, wheat shorts, and corn meal, all on an equal protein basis. The diets were fed to catfish (average weight 5.24 ± 0.04 g) and tilapia (average weight 2.6 ± 0.035 g) over 10 weeks. Each treatment group included four replicate tanks, with 50 fish per tank. After that, fish were evaluated for growth, feed utilization, body proximate, and liver mineral composition. Representative fish were bled for hematological and serological assays and challenged to determine the effects of dietary frass on disease resistance. Final weight gain significantly increased in catfish fed diets containing frass at levels from 10% to 30%. Feed intake of catfish increased with increasing dietary levels of frass. Feed and protein efficiencies, however, were significantly lower in catfish fed frass at levels of 20% and higher compared to the control diet. On the other hand, final weight gain significantly increased in tilapia fed the diet containing the highest level of frass (30%). Tilapia fed diets containing frass (5% to 30%) had significantly higher protein efficiency ratio than the control group. Dietary treatments did not significantly affect feed intake and utilization efficiency of tilapia. Survival, whole-body composition, and mineral content of both fish species were not affected by frass. In catfish, hematological parameters (red blood cell count, hemoglobin, and hematocrit) were improved with the inclusion of frass. Hematological parameters and serum biochemistry of tilapia were unaffected by dietary treatment. In both species, dietary frass enhanced serum alternative complement activity and improved disease resistance from fish pathogenic bacteria. Diets containing frass at levels 20% or more showed significantly higher survival rates against *Flavobacterium covae* than that of control catfish or fish on diets with lower levels of frass. Tilapia fed the diets containing frass showed significant dose-dependent trends in survival against both *F. covae* and *Streptococcus iniae* challenges. Insect frass can be utilized as a feed ingredient to enhance feed palatability, promote the growth of channel catfish and hybrid tilapia, and improve fish's overall resistance to pathogenic bacteria.

Key words: insect larvae frass, alternative feed ingredient, aquaculture nutrition

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Introduction

The growing global population is increasing the demand for animal protein while also generating more waste (Henchion *et al.* 2017). Animal production plays a significant role in this waste generation and resource exploitation. In the United States, farm-raised channel catfish, *Ictalurus punctatus*, represents the largest aquaculture industry, contributing about 75% of the finfish aquaculture (FAO 2022; Hegde *et al.* 2021). Tilapia is also one of the most widely cultured species globally, with a global annual production of 6,100,719t in 2020 (FAO 2022). To keep up with the growth of the aquaculture sector, a well-balanced prepared feed is crucial for achieving high yields and promoting rapid growth at the lowest possible cost (Manam 2023).

To promote sustainable and efficient animal nutrition, insect farming is gaining recognition as a method to mitigate the environmental impacts associated with animal feed production. Fly larvae can be produced economically from low-quality substrates, such as various sources of organic waste, and can be incorporated as a primary protein source in animal feed (Gasco *et al.* 2023; Sogari *et al.* 2019). These characteristics make insects an ideal novel protein source for animal feed formulations while reducing the burden of waste management, promoting a more circular economy, and improving global food security (Diener *et al.* 2009; van Huis *et al.* 2013; Makkar *et al.* 2014). Recent studies indicate that insect-based protein meals can serve as a more sustainable alternative to conventional protein sources, such as fish and plant protein meals, in aquaculture (Prakoso *et al.* 2022; Nunes *et al.* 2023; Islam *et al.* 2024). However, the production of insects results in significant amounts of processing residue, known as “frass” (Gasco *et al.* 2023). Frass, which consists of larval feces, residual larval excrement, and undigested remnants of the substrates, is rich in nutrients (Ravi *et al.* 2020).

Frass is regarded as a biofertilizer that can be used as an alternative to conventional fertilizers for various purposes. It can enhance plant growth and resistance, stimulate seed germination, increase drought and stress tolerance in plants, and support soil microbial communities (Schmitt and de Vries 2020; Barragán-Fonseca *et al.* 2022). Due to its protein, lipid, and bioactive components, along with beneficial microbes (Lopes *et al.* 2022; Mudalungu *et al.* 2021), frass has the potential to serve as an immunomodulatory functional feed ingredient. Thus, the objective of this study was to evaluate the effects of incorporating frass from black soldier fly larvae, *Hermetia illucens*, into the diet at varying levels on the

growth, feed utilization, body composition, hematology, serum biochemistry, immune response, and disease resistance of channel catfish and hybrid tilapia.

Material and Methods

Experimental diets, fish, feeding, and sampling

A nutritionally complete practical basal diet was formulated to contain approximately 31.5% crude protein and 6.2% lipid, based on feedstuff values reported in the NRC (1993) (Table 1). Five experimental diets were prepared, incorporating frass (0%, 5%, 10%, 20%, and 30%) as partial replacements for a combination of soybean meal, wheat shorts, and corn meal, all on an equal protein basis. The frass used in the study was sourced from black soldier flies (*H. illucens*) larvae fed dried distiller's grains and provided by EnviroFlight LLC (Yellow Springs, OH, USA).

Channel catfish (*I. punctatus*) with an average weight of 5.24 ± 0.04 g and hybrid tilapia (Nile x Mozambique, *Oreochromis niloticus* x *O. mossambicus*) fingerlings, averaging 2.6 ± 0.035 g, were randomly stocked into 20 aquaria, each with a volume of 110 L, at a density of 50 fish per aquarium. The aquaria were supplied with flow-through dechlorinated city water, heated to 28°C, and maintained a flow rate of about 0.7 L/min. Each diet was randomly assigned to four aquaria and fed for 10 weeks. Daily feed consumption was recorded. Fish in each aquarium were group-weighted and counted at two-week intervals.

Tissue and blood sampling

At the conclusion of the growth trial, livers from four fish per tank were collected, pooled, and stored at -80°C for subsequent mineral content analysis. The mineral content of the experimental feed and liver samples was measured using a mineral panel analyzed by inductively coupled plasma mass spectrometry (ICP-MS).

At the end of the feeding period, three fish from each tank were anesthetized using tricaine methanesulfonate (MS-222) at 150 mg/L. Blood samples were collected using dried heparinized tuberculin syringes (100 IU) for hematological assays, which included red and white blood cell counts (RBC and WBC), hemoglobin (Hb), and hematocrit (Ht), as described by Yildirim-Aksoy *et al.* (2007). An additional set of fish (4 fish/tank) were bled using non-heparinized tuberculin syringes and allowed to clot and serum samples were collected following centrifugation for the determination of innate immune responses. Lysozyme activity and serum

Table 1 Percentage composition and determined nutrient content of experimental diets

	Experimental diets (%) ¹				
	1	2	3	4	5
Menhaden fish meal	8	8	8	8	8
Soybean meal	45	44	43	41	39
Frass	--	5	10	20	30
Wheat short	24	20.4	16.9	9.8	2.5
Corn meal	14	13.8	13.5	13	12.8
Corn oil	4	3.8	3.6	3.2	2.8
Dicalcium phosphate	1	1	1	1	1
CMC	3	3	3	3	3
Vitamin premix ²	0.5	0.5	0.5	0.5	0.5
Mineral premix ³	0.5	0.5	0.5	0.5	0.5
<u>Proximate composition (%) -catfish</u>					
Dry matter	91.68	91.68	91.85	91.78	91.56
Protein	31.80	31.28	31.77	31.83	31.51
Lipid	6.25	6.15	6.33	6.11	6.01
Ash	7.20	7.32	7.74	8.26	8.81
<u>Proximate composition (%) -tilapia</u>					
Dry matter	91.68	91.68	91.85	91.78	91.56
Protein	31.60	31.00	31.20	31.10	31.00
Lipid	5.28	5.52	5.71	5.56	5.49
Ash	6.80	6.97	7.16	7.39	7.68

CMC, carboxymethyl cellulose; Frass is by-product of black soldier fly (*H. illucens*) larva meal industry.

¹ Diets 1, 2, 3, 4, and 5 contained 0, 5, 10, 20, and 30% frass, respectively.

² Vitamin premix, diluted in cellulose, provided by following vitamins (mg/kg diet): vitamin A (520,400 IU/g), 7.7; vitamin D3 (108,300 IU/g), 18.5; vitamin E (250 IU/g), 200; vitamin K, 10; thiamin, 10; riboflavin, 12; pyridoxine, 10; calcium pantothenate, 32; nicotinic acid, 80; folic acid, 2; vitamin B12, 0.01; biotin, 0.2; choline chloride, 400; and L-ascorbyl-2-polyphosphate (35% vitamin C activity), 60.

³ Trace mineral premix provided by following minerals (mg/kg diet): zinc (as ZnSO₄·7H₂O), 150; iron (as FeSO₄·7H₂O), 40; manganese (as MnSO₄·7H₂O), 25; copper (as CuCl₂), 3; iodine (as KI), 5; cobalt (as CoCl₂·6H₂O), 0.05; and selenium (as Na₂SeO₃), 0.09.

natural hemolytic (alternative pathway) complement activity were performed as described in Yildirim-Aksoy *et al.* (2007). Lysozyme activity in serum involved measuring the lytic activity of catfish serum against the bacterium *Micrococcus lysodeikticus* (Sigma, St. Louis, MO, USA). The complement assay relied on the hemolysis of sheep erythrocytes (Remel Inc., Lenexa, KS, USA) by complement present in serum.

Bacterial Challenge

To utilize a common garden approach, at the end of the feeding period, 30 (tilapia) and 15 (catfish) of the remaining fish in each aquarium were tagged with Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Inc., Shaw Island, WA, USA). The tagged fish were distributed into new aquaria, each housing a total of 30 fish (six fish each from all five dietary treatments, resulting in 30 fish per aquarium for challenge tests).

Then, the tagged catfish or tilapia in the 10 aquaria (totaling 60 fish per dietary treatment) were exposed to predetermined

concentrations of *Flavobacterium covae* through immersion at 1 x 10⁹ cells/mL and 2 x 10⁵ cells/mL, respectively. Another set of tagged tilapia in the 10 aquaria (60 fish/dietary treatment) were injected with a predetermined concentration of *Streptococcus iniae* at 1 x 10⁴ cells/ml concentration. During this period, the fish were fed the control diet and monitored twice daily, recording and removing any moribund or dead fish for seven days.

Statistical analysis

Data were analyzed using one-way ANOVA with the general linear model. Comparisons of treatment means were conducted using Tukey's multiple comparison test in GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). The trends for mean responses across different levels of dietary larval frass, as well as weight gain and feed utilization parameters, were analyzed through orthogonal polynomial contrasts using SAS software version 9.4 (SAS Institute, Cary, NC, USA). A significance level of 0.05 or below was considered statistically significant.

Results

Growth performance, feed utilization, and survival are given in Table 2. Dietary frass at 10% significantly ($p < 0.0026$) increased the weight gain of catfish without affecting feed and protein efficiencies. Weight gain and feed intake were linearly increased, whereas feed efficiency (FER), protein efficiency ratio (PER), and apparent protein utilization (APU) were linearly decreased with increasing levels of dietary frass. Fish fed dietary frass at a level of 20% had the highest weight gain but significantly reduced the FER, PER, and APU. Including frass at a level of 30% significantly enhanced the weight of tilapia. Fish fed diets containing frass (5 % to 30 %) had significantly higher PER than the group fed a diet without frass (control diet). Fish fed diets with frass also showed improved APU, but it was only significantly higher in fish fed the diet with 30% frass than that of the control fish. Weight gain ($p < 0.0048$), PER ($p < 0.0091$), and APU ($p < 0.0395$) linearly increased with increasing levels of dietary larval frass (Table 2).

The liver concentration of iron increased incrementally with increasing concentrations of dietary frass, but the values

were significantly higher only at the highest dietary frass level (30 %) (Table 3). The inclusion of frass resulted in the improvement of hematological (red blood cell (RBC) count, hemoglobin, and hematocrit) parameters of catfish (Fig.1). The RBC count of fish fed with a 10% or higher frass diet was significantly ($p < 0.01$) higher than that of the control fish. Hemoglobin concentration and hematocrit increased at each incremental level of dietary frass and significantly ($p < 0.05$) higher in catfish fed a 30 % frass diet. Dietary levels of frass did not influence the hematological values of tilapia. Serum complement activities increased in both catfish and tilapia fed frass diets (data not shown).

In the challenge tests, catfish that had been fed diets containing 20 % and 30 % frass and tilapia that had received 10% and 30% frass demonstrated significantly better survival rates ($p < 0.05$) than those on lower frass levels and the control diet. Additionally, tilapia that had been fed diets with 30% frass showed improved survival rates when challenged with *S. iniae* (Fig.2). The post-challenge survival rates from both *F. covae* ($R^2 = 0.699$) and *S. iniae* ($R^2 = 0.881$) showed a linear response to the dietary frass levels.

Table 2 Weight gain, feed intake, FER, PER, APU, and survival of channel catfish and hybrid tilapia (Nile x Mozambique) fed diets containing various level of frass for 10 weeks ¹

	Dietary levels of frass (%)					P-value	Linear (Pr > F)
	0	5	10	20	30		
Channel catfish							
Weight gain (g)	21.72	22.15	24.34*	25.49**	25.01**	0.0026	0.0002
Feed intake (g)	28.03	30.39*	32.92***	34.86****	35.35****	<0.0001	<0.0000
FER ²	0.78	0.74	0.74	0.73*	0.71***	0.0017	0.0005
PER ³	2.44	2.37	2.33	2.30	2.25**	0.0039	0.0002
APU (%) ⁴	39.70	37.80	37.20	36.60*	35.60**	0.0039	0.0002
Survival (%)	98.5	97.3	98	99	100	0.3391	0.2105
Hybrid tilapia							
Weight gain (g)	48.97	51.26	51.81	51.88	55.26*	1.265	0.0048
Feed intake (g)	59.81	60.29	60.85	60.58	64.94	1.517	0.0483
FER ²	0.82	0.85	0.85	0.85	0.85	0.011	0.0579
PER ³	2.58	2.72*	2.73*	2.73*	2.74*	0.034	0.0091
APU (%) ⁴	39.24	41.4	41.23	41.21	41.94*	0.695	0.0395
Survival (%)	92.35	88.78	87.24	93.88	84.69	3.666	0.3978

FER, feed efficiency ratio; PER, protein efficiency ratio; APU, apparent protein utilization.

¹ Values are means of four replicates per treatment. Asterisks indicate significant difference between the control and frass fed groups. Number of asterisks represent degree of statistically significant difference from control: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

² FER = weight gain (g)/dry feed fed (g).

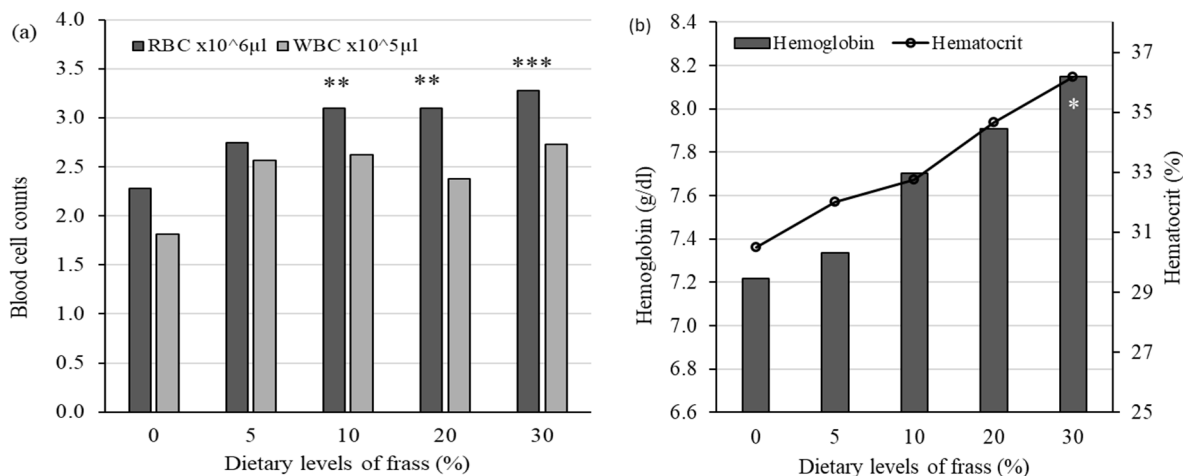
³ PER = wet weight gain (g)/crude protein fed (g).

⁴ APU = 100 x [body protein gain (g)/crude protein fed (g)].

Table 3 Liver mineral analysis (dry matter basis) of channel catfish fed diets containing various levels of frass for 10 weeks¹

	Dietary levels of frass (%)					P-value
	0	5	10	20	30	
Macro minerals (mg g ⁻¹ dry liver)						
Calcium	0.27	0.28	0.26	0.27	0.27	0.7265
Potassium	8.6	7.98	8.45	8.29	8.53	0.6579
Magnesium	0.58	0.57	0.58	0.54	0.57	0.2036
Phosphorus	7.38	7.52	7.12	7.23	7.35	0.731
Sulfur	0.11	0.11	0.1	0.1	0.11	0.6453
Trace minerals (µg g ⁻¹ dry liver)						
Manganese	2.9	2.8	3.43	2.98	3.14	0.0826
Copper	18.32	17.35	18.25	16.16	15.75	0.3415
Iron	64.28	69.65	69.71	80.46	96.8*	0.0103
Aluminum	2.73	2.8	2.58	2.19	2.74	0.8243
Zinc	99.05	99.15	98.47	91.73	93.99	0.5754

¹ Values are means of two determinations of pooled liver samples of four fish per tank and four tanks per treatment. Asterisks denote statistically significant differences at $p < 0.05$.

**Fig.1** Hematological values of channel catfish fed diets containing various level of frass for 10 weeks

RBC, red blood cell count; WBC, white blood cell count; Hb, hemoglobin; Ht, hematocrit. Values are means of one determination per fish, four fish per tank and four tanks per treatment. Asterisks indicate significant difference between the control and frass fed groups. Number of asterisks represent degree of statistically significant difference from control: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Discussion

Our research indicates that while larval frass was efficiently utilized by both juvenile channel catfish and hybrid tilapia, its effectiveness on catfish performance is limited. After 10 weeks on a diet containing 10% or more frass, the catfish's weight increased significantly. This growth improvement was associated with a significant increase in feed intake. However, increasing the frass content from 20% to 30% did not lead to further growth benefits; it reduced feed and protein efficiency. This indicates that a careful balance is necessary when

incorporating frass into fish diets. Similarly, improvements in both catfish and plant growth have been observed in aquaponic systems that used dietary frass at a ratio of 10% (Romano *et al.* 2023). The authors suggested that the increased growth in catfish may be linked to the upregulation of genes associated with growth, a reduction in intestinal inflammation, and a significant enhancement in feed intake.

For tilapia, including frass at a 30% level significantly boosted their weight. Although no significant differences were noted in feed intake or feed efficiency ratio (FER) among fish fed diets with varying frass levels, there was a

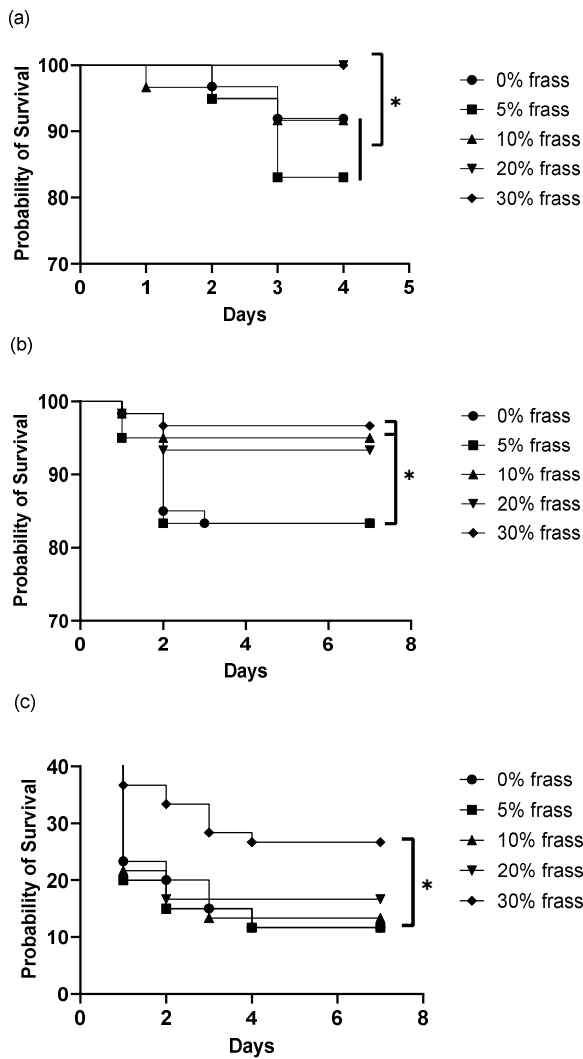


Fig.2 Percent survival of channel catfish challenged with *Flavobacterium covaе*, ALG-00-053 (a) and hybrid tilapia (Nile x Mozambique) challenged with *F. covaе*, ALG-00-053 (b) and *Streptococcus iniae* #60 (c) previously fed various level of frass for 10 weeks

Values are means of 60 fish per treatment. Asterisks indicate significant difference in the survival curves between the control and frass fed groups at $p < 0.05$.

noticeable trend of improvement with the inclusion of frass. Fish consuming diets with frass (ranging from 5% to 30%) displayed significantly higher protein efficiency ratios (PER) compared to the no-frass group. Furthermore, the fish that consumed frass-rich diets showed improved apparent protein utilization (APU), with a significant increase observed in fish fed the 30% frass diet compared to the control group. There were no significant differences in the whole-body proximate composition of catfish or tilapia across the different frass diet levels (5% to 30%) (Yildirim-Aksoy *et al.* 2020a, 2020b).

Similarly, Romano *et al.* (2024) fed Mozambique tilapia (*O. mossambicus*) a diet consisting of 10% frass from larvae that were fed an expired fish diet (45% crude protein) over 8 weeks, which significantly enhanced tilapia growth compared to those fed the control diet.

These findings suggest hybrid tilapia seem more efficient than channel catfish in converting ingested frass feed into fish body mass. This increased efficiency may be due to hybrid tilapia's higher chitinase activity, which allows them to better degrade chitin compared to catfish. Removing chitin from insect meal has been shown to improve the digestibility of insect protein (Bonomini *et al.* 2024). This improvement occurs because chitin is a complex matrix of proteins, lipids, and other components. The hydrolysis of chitin enhances the accessibility of digestive enzymes to proteins and lipids, thereby increasing protein digestibility (Bonomini *et al.* 2024). Additionally, the poorer FER observed with higher levels of frass in the catfish diets may be due to the higher ash content of the frass used in catfish diets compared to the tilapia study (Table 1).

Including frass has shown promise as a beneficial dietary supplement, enhancing fish's hematological and innate immune parameters. A significant increase in complement activity was observed in both catfish and tilapia fed diets with frass (Yildirim-Aksoy *et al.* 2020a, 2020b). Additionally, Sankappa *et al.* (2024) reported changes in global gene expression and activation of innate and adaptive immunity in channel catfish that consumed diets containing frass. Furthermore, a diet including 20% frass in Pacific white shrimp (*Litopenaeus vannamei*) significantly increased the serum's ability to inhibit the growth of *Vibrio parahaemolyticus* (Yildirim-Aksoy *et al.* 2022). In yellow catfish (*Pelteobagrus fulvidraco*), enhanced immune responses were also noted when fish meal protein in the diet was substituted with 13-48% black soldier fly larvae meal protein (Xiao *et al.* 2018). Including insect meal in the Nile tilapia diet improved skin, mucus lysozyme, and peroxidase activities (Tippayadara *et al.* 2021). A related study on red seabream (*Pagrus major*) found that incorporating low doses (0.75% and 7.5%) of housefly (*Musca domestica*) pupae into their diet for ten days resulted in a significant enhancement of the phagocytic activity of peritoneal macrophages (Ido *et al.* 2015). Notably, fish that received 5% dietary housefly pupae for two months experienced 100% survival against the bacterial pathogen *Edwardsiella tarda*, while all control fish perished within 12 days of the bacterial challenge (Ido *et al.* 2015). In the present study, catfish fed diets containing 20% and 30% frass

exhibited better survival rates following challenges with *F. covae* than those on the control diet or diets with lower levels of frass. Similarly, tilapia that received a diet with 30% frass showed a significant increase in survival against challenges from both *F. covae* and *S. iniae*. In another study, small European sea bass (*Dicentrarchus labrax*) fed *Tenebrio molitor* larvae meal for six weeks showed anti-inflammatory responses, including increases in ceruloplasmin, myeloperoxidase, and nitric oxide levels (Henry *et al.* 2018).

The positive responses observed could be linked to the bioactive components found in insect frass. Various components derived from insects, such as antimicrobial peptides (AMPs), lauric acid, beneficial microbes, and chitin, can serve as immunomodulatory functional feed ingredients (Koutsos *et al.* 2022). Insects are among the richest sources of AMPs (Yi *et al.* 2014; Vogel *et al.* 2018; Silveira *et al.* 2021). Research has demonstrated that AMPs are effective in killing bacteria and controlling pathogen infections in animals including fish (Rodrigues *et al.*, 2021; Silveira *et al.*, 2021; Wang *et al.*, 2022). Insect meal and frass are rich sources of chitin, which can activate the immune system in mammals (O'Neil *et al.* 2006). The benefits of dietary chitin and/or chitosan have been well-documented in fish and shellfish (Harikrishnan *et al.* 2012; Gopalakannan and Arul 2006). Furthermore, chitin may act as a prebiotic, being non-digestible by the host, while selectively promoting the growth of beneficial intestinal microbiota that could help prevent the growth and colonization of pathogenic bacteria (Mousavi *et al.* 2020; Sankian *et al.* 2018).

In conclusion, frass generated from the production of black soldier fly larvae has the potential to be used as a feed ingredient that can increase feed intake and improve fish growth. Although frass may not be an ideal dietary component for carnivorous fish (Kagata and Ohgushi 2012; Banavar *et al.* 2022), incorporating it into the diets of omnivorous fish, especially at higher levels, could provide benefits such as boosting innate immune components and enhancing fish resistance to bacterial infections. In addition to its environmental advantages, converting organic waste into a high-value feed ingredient could offer significant economic benefits.

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Annotated Bibliography of Key Works

(1) Romano N, Datta SN, Pande GSJ, Sinha AK, Yamamoto FY, Beck BH, Webster CD (2023) Dietary inclusions of black soldier fly (*Hermetia illucens*) larvae frass enhanced production of channel catfish (*Ictalurus punctatus*) juveniles, stevia (*Stevia rebaudiana*), and lavender (*Lavandula angustifolia*) in an aquaponic system. *Aquaculture*, **575**, 739742. <https://doi.org/10.1016/j.aquaculture.2023.739742>

For the first time, the authors explored the potential of frass to enhance fish and plant production using a dietary approach in an aquaponic system. Channel catfish (*Ictalurus punctatus*) juveniles were fed with or without 10% black soldier fly (*Hermetia illucens*) larvae frass for 8 weeks. The authors used a 2 x 2 factorial design, with the main effects of dietary frass inclusion and media type at two different plant bed types. Overall, the authors reported improved fish and plant growth in an aquaponic system with dietary frass. The authors suggested that increased catfish growth was likely due to the upregulation of genes responsible for growth, reduction in intestinal inflammation, and a significant enhancement in feed intake. Additionally, it was noted that dietary frass contributed more water-borne nutrients for the plants, resulting in better plant growth.

(2) Romano N, Yamamoto F, Rawles SD, Webster CD (2024) Type of black soldier fly (*Hermetia illucens*) larvae frass influences the nutritional value when included in a prepared diet for Mozambique tilapia (*Oreochromis mossambicus*). *Aquaculture*, **589**, 740946. <https://doi.org/10.1016/j.aquaculture.2024.740946>

The authors, for the first time, present the nutritive value of frass from black soldier larvae fed on different substrates for Mozambique tilapia (*Oreochromis mozambicus*). They hypothesized that the initial substrate would influence the composition of frass and, thus, the nutritive values for fish. The study examined frass from larvae fed either an expired fish diet (45% crude protein) or a combination of fruits/vegetable

peels (9.3 % crude protein) at 5 % and 10 % dietary inclusion levels over 8 weeks. They showed that expired fish diet-based frass at 10 % significantly enhanced tilapia growth compared to fish fed the control diet. Additionally, the authors provided histological scoring for the liver and intestine, noting mild liver inflammation but improved intestinal histomorphology. The findings suggest that the initial substrate fed to black soldier fly larvae has important implications for the nutritional value of their frass and the associated liver and intestinal health of the fish consuming it.

(3) Yildirim-Aksoy M, Eljack R, Beck BH, Peatman E (2022) Nutritional evaluation of frass from black soldier fly larvae as potential feed ingredient for Pacific white shrimp, *Litopenaeus vannamei*. *Aquac. Rep.*, **27**, 101353. <https://doi.org/10.1016/j.aqrep.2022.101353>

For the first time, the authors present the nutritional value and health benefits of frass derived from the larvae of black soldier flies fed dried distillers' grains in shrimp. They examined the inclusion of frass at levels up to 30 % in the diets of Pacific white shrimp, *Litopenaeus vannamei*. Overall, the authors showed a quadratic growth trend with increasing dietary levels of frass, with 5 % frass at the highest and 30 % frass at the lowest weight gain. Feed efficiency and body composition were also provided. Additionally, the authors assessed the health benefits of incorporating dietary frass by analyzing serum samples for immune parameters and antibacterial activities. The findings highlight the implications of feeding shrimp different levels of frass, which are discussed in detail in this paper.

(4) Banavar A, Amirkolaei SK, Duscher L, Khairunisa BH, Mukhopadhyay B, Schwarz M, Urick S, Ovissipour R (2022)

Nutritional evaluation of black soldier fly frass as an ingredient in Florida pompano (*Trachinotus carolinus* L.) diets. *Animals (Basel)*, **12**, 2407. <https://doi.org/10.3390/ani12182407>

The authors examined the potential of using frass as a dietary ingredient for a carnivorous fish species for the first time. They evaluated the impact of three different levels of dietary frass -8 %, 12 %, and 18 % -on the growth performance and feed utilization of Florida pompano (*Trachinotus carolinus* L.). The findings suggest that pompano cannot fully utilize frass, resulting in decreased growth performance as the level of frass increases. Additionally, the authors analyzed the intestinal microbiome, which revealed the highest diversity of gut flora in the control diet, while diets containing frass displayed signs of community imbalance. The authors concluded that frass is not an ideal dietary component for carnivorous fish.

(5) Sankappa NM, Lange MD, Yildirim-Aksoy M, Eljack R, Kucuktas H, Beck BH, Abernathy JW (2024) Transcriptome analysis and immune gene expression of channel catfish (*Ictalurus punctatus*) fed diets with inclusion of frass from black soldier fly larvae. *Front. Physiol.*, **14**, 1330368. doi: 10.3389/fphys.2023.1330368

The authors reported systemic and mucosal tissue gene expression, especially regarding the growth and immune-related genes of channel catfish (*Ictalurus punctatus*) fed various levels of frass for 10 weeks. They examined liver, head, kidney, gill, and intestine samples for gene expression analyses. Further, they identified differential expression of genes using targeted quantitative PCR panels for both innate and adaptive immune genes from channel catfish. Overall, the authors showed alteration of global gene expression and activation of innate and adaptive immunity of channel catfish fed diet with frass.