

# Nutritive and Microbiological Quality of Fish Feed Formulated from Local Flours Enriched with Larvae of *Hermetia illucens*

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## Abstract

In aquaculture, feed represents the main component of production costs, and the development of this sector depends on the development of an economical feed formulation that meets the qualitative and quantitative requirements of fish. The aim of this study was to determine the nutritive and microbiological quality of fish feed formulated from local flours enriched with *Hermetia illucens* larvae. The raw materials used for formulation were fishmeal, corn meal, low-grade rice, soybean meal and *Hermetia illucens* larvae meal. Different iso-protein feed compositions were prepared with 0%, 10%, 25%, 35%, 50%, 65%, 75% and 100% incorporation of *Hermetia illucens* larvae meal as a substitute for fish meal. Biochemical and microbiological analyses of these flours were determined using standard methods. The results showed that incorporation of larvae meal had an influence on the biochemical characteristics ash (8.15 to 20.27%), lipid (11.55 to 24.94%), fiber (13.93 to 20.41%) and dry matter (89.65 to 91.19%) of various formulated feed. Loads of fecal Streptococci, Staphylococci, *Aeromonas*, yeasts and molds ranged from 2.4 to 4.9 log<sub>10</sub> CFU/g; 3.6 to 3.9 log<sub>10</sub> CFU/g; 2.2 to 2.7 log<sub>10</sub> CFU/g; 2.1 to 2.3 log<sub>10</sub> CFU/g, respectively. The level of contamination of these flours was below the microbiological criteria applicable to animal feed. Feed formulated with 0% and 10% *Hermetia illucens* larvae showed the best nutritive and microbiological characteristics. These results suggest that flours enriched with *Hermetia illucens* larvae could be used in fish feed.

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## Keywords

*Hermetia illucens*, Fish Feed, Nutritive and Microbiological Quality

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## 1. Introduction

Formulating an appropriate diet is one of the most important factors in commercial aquaculture, as feed production costs and the associated impact on fish growth are major expenses [1]. Fish meal is considered the main protein source used in aquaculture feeds formulation due to its abundant amino acid composition, superior digestibility and palatability [2]. However, in recent years, the availability of fish meal has been limited due to overexploitation of marine resources, expressively fished for fish meal and fish oil production. What's more, from an ethical and moral point of view, these high-quality foods could be better directed directly to human consumption [3]. This situation has made it possible to find an alternative to marine products through the use of plant-based raw materials [4]. However, plant proteins cannot replace higher quantities of fishmeal due to the presence of anti-nutritional factors, high crude fiber content and an unbalanced amino acid profile [5]. High substitution rates lead to significant reductions in performance (growth, even survival) in farmed species with high trophic levels and high protein and lipid requirements [4]. In the search for new sources of ecologically sustainable feed, numerous studies have demonstrated the alternative role that insects could play in animal nutrition. Insects, and in particular the black soldier fly (*Hermetia illucens*), thus appear to be one of the most interesting new sources of protein [6]. Indeed, black soldier fly larvae (BSF, *Hermetia illucens*) stand out from other flies because of their ability to be reared on a wide range of organic matter, their relatively short life cycle [7] and their high protein (42%) and fat (29%) content [8]. The larvae of the fly *Hermetia illucens* are an alternative source of protein and energy for animal feed. Previous research has demonstrated that these larvae can be used for aquaculture feed. These larvae also offer the opportunity to introduce a circular economy model through the valorization of residual organic matter to produce feed that is, in turn, used in animal feed [9]. This is a promising alternative to the usual sources of animal protein, such as fish meal, used in animal feed. However, given their origin in residual organic matter, larvae could present numerous health risks due to the possible presence of more or less toxic contaminations and pathogens. The aim of this study was to determine the nutritive and microbiological quality of fish feed formulated from local flours enriched with *Hermetia illucens* larvae.

## 2. Material and Methods

### 2.1. Material

The biological material consisted of *Hermetia illucens* larvae obtained after culturing black soldier flies (BSF) and flours of corn (*Zea mays*), soybean meal

(Glycine max), fish and low-grade rice (*Oryza sativa*). The various flours were purchased from feed suppliers in municipalities of Port-Bouët, Bingerville and Yopougon, in the district of Abidjan, Côte d'Ivoire.

## 2.2. Methods

### 2.2.1. Preparation of Black Soldier Fly (*Hermetia illucens*) Larvae Meal

To obtain the flour, the larvae collected from the farms were rinsed with sterile distilled water. They were then scalded for 1 minute by direct immersion in a 100°C water bath and vacuum-packed in plastic bags for freezing at -40°C. For drying, the larvae were thawed at 4°C in the refrigerator overnight, and dried in an electric oven at 60°C for 48 - 72 h. After drying, the larvae were ground to meal using a grinder (IKA Labortechnik), then sieved using a 500 µm stainless steel sieve, washed and autoclaved (SYSTEC) at 121°C for 15 min. The flour obtained was packaged in a 1 L sterile plastic bowl.

### 2.2.2. Preparation of Local Flours

The various local flours purchased with the local retailers were sieved using a 500 µm mesh stainless steel sieve previously washed and sterilized in an autoclave at 121°C for 15 min, and packaged in sterile liter plastic bowls.

### 2.2.3. Feed Formulation Method

The formulation method used to produce fish feeds is the linear programming method used by several authors [10]. This method makes it possible to compare a wide range of raw materials in order to determine the quantities of inputs that provide the desired nutrient levels at the lowest possible cost (Table 1).

The formulation method made it possible to track variations in protein, lipid, ash and fiber content as raw material quantities varied. Depending on the formulation targets set, the different quantities of raw materials taken were determined. The formulation targets for our study are:

- Sum of protein percentages  $\Sigma P = 38\%$ ;
- Substitute fish meal for larvae meal at 0%, 10%; 25%; 35%; 50%; 65%; 75% and 100%.

**Table 1.** Formulation table for experimental foods.

Raw materials	Quantity	Proteins	Lipids	Ashes	Fibers
RM1	Q1	P1 = Q1 × % P1	L1 = Q1 × % L1	C1 = Q1 × % C1	F1 = Q1 × % F1
RM2	Q2	P2 = Q2 × % P2	L2 = Q2 × % L2	C2 = Q2 × % C2	F2 = Q2 × % F2
RM3	Q3	P3 = Q3 × % P3	L3 = Q3 × % L3	C3 = Q3 × % C3	F3 = Q3 × % F3
RMn	Qn	Pn = Qn × % Pn	Ln = Qn × % Ln	Cn = Qn × % Cn	Fn = Qn × % Fn
Total	$\Sigma Q = 100$	$\Sigma P$	$\Sigma L$	$\Sigma C$	$\Sigma F$

With RM1 = Raw material 1; Q1 = Quantity of RM1; P1 = Quantity of RM1  $\times$  percentage of proteins of RM1; L1 = Quantity of RM1  $\times$  percentage of lipids of RM1; C1 = Quantity of RM1  $\times$  percentage of ashes of RM1; F1 = Quantity of RM1  $\times$  percentage of Fibers of RM1; P1 = Quantity of proteins brought by RM1; L1 = Quantity of lipids provided by RM1; C1 = Quantity of ash provided by RM1; F1 = Quantity of fiber provided by RM1;  $\Sigma P$  = Protein rate of the formulated feed;  $\Sigma L$  = Lipid rate of the formulated feed;  $\Sigma C$  = Ash rate of the formulated feed;  $\Sigma F$  = Fiber rate of the formulated feed.

#### 2.2.4. Feed Formulation

BSF larvae powder was added to the fish feed composed of fish meal, corn meal, low-grade rice meal and soybean meal. Several fish feeds have been formulated by incorporating BSF larvae powder at 0%, 10%, 35%, 50%, 65%, 75% and 100% in place of fish meal. These feeds are produced in four stages. The ingredients are first sieved (500  $\mu\text{m}$  mesh) to remove coarse particles, then mixed and blended to homogenize the mixture, and finally granulated and dried. These feeds were prepared using an automatic calculation system based on the linear programming formulation method [11]. The feeds were formulated under hygienic conditions.

#### 2.2.5. Biochemical Analysis of Ingredients and Formulated Feeds

All ingredients in the formulated feeds were analyzed individually after purchase from the feed suppliers and then after formulation. The dry matter, ash, protein, fat and fiber contents of the various formulated feeds were determined using the AOAC [12] method. The dry matter content of each sample was determined using an electronic moisture meter (OHAUS) at 105°C for 45 min. Ash content was obtained by calcining the sample in a muffle furnace (Nabertherm 30 - 3000°C) at 550°C for 24 hours. Protein content was determined using the Kjeldahl method [12] for total nitrogen ( $N \times 6.25$ ). Total lipids were determined by the Soxhlet method [13] (using hexane as solvent). Fiber content was obtained by acid hydrolysis of the samples [12].

#### 2.2.6. Microbiological Analysis of Ingredients and Formulated Feeds

The microbiological analysis consisted of counting indicative faecal contamination bacteria, such as *E. coli*, Anaerobic Sulfite-Reducing, faecal Streptococci, and also yeasts, molds, searching for pathogenic bacteria such as *Salmonella* and potentially pathogenic *Staphylococcus*, *Pseudomonas*, *Aeromonas* and *Vibrio*. Enumeration of microbial flora associated with flours was carried out using the standard method. For analysis, stock suspensions were prepared by adding 10 g of each feed sample to 90 ml of buffered peptone water (BPW) (Scharlau, France). The suspensions were homogenized for one minute, and several decimal dilutions were made from the mother suspension. Yeasts and molds were counted according to ISO 21527-1, 2008 on Sabouraud medium (Pan Reac Applichem). After culture, incubation lasted 3 to 5 days at 25°C. Yeast colonies were round, smooth, whitish and creamy. As for the mold colonies, they were of different colors,

depending on the type of mold. Anaerobic Sulfite-Reducing (ASR) were enumerated according to ISO 7937: 2004 on TSN (Tryptone sulfite neomycin) medium (Sharlau, France). Incubation was at 37°C for 24 hours. Small and large black colonies were counted. *Escherichia coli* was counted according to NF ISO 16649-2 on Rapid *E. coli* 2 medium incubated at 44°C for 24 h. Characteristic colonies were S-type and purple in color. Faecal Streptococci were counted in accordance with ISO 7899-2:2000 on Bile Esculin Azide agar (Thermo Fisher, USA). Incubation was at 37°C for 24 h. Small translucent colonies with a black halo were counted. Staphylococci were counted using the ISO 6888 method on Baird Parker agar (Conda, Spain) supplemented with egg yolk and potassium tellurite (Bio-Rad, France). Incubation took place at 37°C for 24 h. Only characteristic black colonies with a clear halo and an opaque zone around it were taken into account. A confirmatory test was then carried out for Staphylocoagulase using oxalated rabbit plasma, in accordance with standard NF V08-057-1 (January 2004). *Vibrio* enumeration was performed according to ISO 21872-1, 2017 on TCBS (Thiosulfate, Citrate, Bile, Sucrose) medium (Oxoid, UK). Incubation took place at 37°C for 24 h. After incubation, colonies were medium-sized, smooth, opaque, yellow or green. *Aeromonas* enumeration was performed on Aeromonas Isolation Agar. Incubation was at 37°C for 24 h. After incubation, yellow colonies were counted. *Pseudomonas* was counted on Cetrimide agar (Scharlau, France). Incubation was carried out at 42°C for 24 h. Blue-green, yellow-green and brown-red colonies were counted. *Salmonella sp.* was detected according to ISO 6579-1: 2017 in 4 steps: pre-enrichment, selective enrichment, isolation and identification by biochemical characters.

### 2.2.7. Determining the Microbiological Quality of Formulated Feeds

The microbiological quality of formulated foods was determined on the basis of the microbiological criteria set out in ISO 11133: 2014.

For *Salmonella*, results are interpreted on the basis of the 2-class plan [14].

For the other germs, results were interpreted according to a 3-class plan, following the m reference criteria [14].

### 2.2.8. Expression of Results

The results obtained were expressed in colony-forming units (CFU)/g by applying the formula:

$$N = \frac{\sum \text{colonies}}{V_{mi} * (n_1 + 0.1n_2) * d_1}$$

Où:	nombre d'UFC par gramme de produit initial;
$N$	somme des colonies des boîtes interprétables;
$\sum \text{colonies}$	volume de solution déposée;
$V_{mi}$	nombre de boîtes considéré à la première dilution retenue;
$n_1$	nombre de boîte considéré à la seconde dilution retenue;
$n_2$	facteur de la première dilution retenue;
$d_1$	nombre d'UFC par gramme de produit initial.

### 2.2.9. Statistical Analyses

The analysis of variance (ANOVA) was performed with the R 4.3.2 software to study the degree of difference between the variables. In the event of a significant difference between the studied parameters, the classification of the means (homogeneous groups) is carried out with the Turkey test. The significance threshold ( $\alpha$ ) is 0.05. Statistical differences with a probability value of less than 0.05 were considered significant. The values used for the realization of the histograms on Excel, were in log10, while those used for the realization of the tables were in CFU/g of formulated foods.

## 3. Results

### 3.1. Biochemical Composition of Larvae Flour and Local Flours

Results of biochemical analyses of larvae, fish, corn, soybean flour and poor rice flour are presented in **Table 2**. These results show that there is a significant difference ( $p < 0.05$ ) between the different flours in terms of the parameters sought. Protein, lipid, ash, dry matter and fiber contents varied respectively from 7.98% to 46.84%; 1.30% to 22.96%; 2.65% to 25.03%; 88.80% to 96.51% and 2.80% to 15.09%. According to the results, black soldier fly larvae meal had high levels of dry matter (96.51%), lipids (22.96%) and total fiber (15.09%), while fish meal had the highest levels of protein (46.84%) and ash (25.03%). Corn flour and soybean flour were low in protein (7.98%), ash (2.65%), dry matter (88.80%) and total fiber (2.80%) in the case of corn flour, and in fat (1.30%) in the case of soybean flour.

**Table 2.** Biochemical characteristics of larvae and local meals.

	Dry matter (%)	Ash (%)	Protein (%)	Lipid (%)	Fiber (%)
<b>Larva</b>	96.51 ± 0.23 <sup>a</sup>	9.32 ± 0.58 <sup>b</sup>	46.04 ± 0.01 <sup>b</sup>	22.96 ± 0.01 <sup>a</sup>	15.09 ± 0.13 <sup>a</sup>
<b>Fish</b>	91.55 ± 0.19 <sup>b</sup>	25.03 ± 0.04 <sup>a</sup>	46.84 ± 0.13 <sup>a</sup>	11.46 ± 0.28 <sup>c</sup>	9.52 ± 0.27 <sup>b</sup>
<b>Soybean</b>	90.18 ± 0.10 <sup>c</sup>	6.76 ± 0.06 <sup>c</sup>	43.74 ± 0.12 <sup>c</sup>	1.30 ± 0.02 <sup>e</sup>	4.40 ± 0.16 <sup>c</sup>
<b>Corn</b>	88.80 ± 0.01 <sup>d</sup>	2.65 ± 0.06 <sup>d</sup>	7.98 ± 0.01 <sup>e</sup>	3.85 ± 0.00 <sup>d</sup>	2.80 ± 0.28 <sup>d</sup>
<b>Rice</b>	91.22 ± 0.11 <sup>b</sup>	9.06 ± 0.01 <sup>b</sup>	12.84 ± 0.06 <sup>d</sup>	14.78 ± 0.03 <sup>b</sup>	9.97 ± 0.37 <sup>b</sup>

### 3.2. Biochemical Composition of Formulated Feeds

Formulated feeds with 0%, 10%, 25%, 35%, 50%, 65%, 75% and 100% incorporation of BSF larvae meal showed a significant difference ( $p < 0.05$ ) in the parameters sought, except for crude protein ( $p$ -value  $> 0.05$ ). Dry matter varied from 89.65% to 91.19%. Ash varied from 20.27% to 8.15%. Total fiber and lipid ranged from 13.93% to 20.41% and 11.55% to 24.94% respectively (**Table 3**).

**Table 3.** Biochemical characteristics of formulated feeds.

	Feed percent Progress							
	0%	10%	25%	35%	50%	65%	75%	100%
<b>Dry matter</b>	89.65 ± 0.07 <sup>e</sup>	91.19 ± 0.11 <sup>a</sup>	90.99 ± 0.01 <sup>ab</sup>	90.87 ± 0.07 <sup>abc</sup>	90.67 ± 0.05 <sup>bcd</sup>	90.50 ± 0.10 <sup>d</sup>	90.57 ± 0.07 <sup>cd</sup>	91.03 ± 0.16 <sup>a</sup>
<b>Ash</b>	20.27 ± 0.34 <sup>a</sup>	19.07 ± 0.09 <sup>b</sup>	17.16 ± 0.16 <sup>c</sup>	15.23 ± 0.03 <sup>d</sup>	12.78 ± 0.01 <sup>e</sup>	11.50 ± 0.14 <sup>f</sup>	9.96 ± 0.04 <sup>g</sup>	8.15 ± 0.03 <sup>h</sup>
<b>Protein</b>	39.14 ± 0.85 <sup>a</sup>	39.35 ± 1.01 <sup>a</sup>	39.22 ± 0.78 <sup>a</sup>	38.67 ± 1.04 <sup>a</sup>	38.74 ± 0.80 <sup>a</sup>	37.79 ± 0.78 <sup>a</sup>	37.16 ± 1.37 <sup>a</sup>	36.81 ± 1.80 <sup>a</sup>
<b>Lipid</b>	11.55 ± 0.04 <sup>h</sup>	13.03 ± 0.03 <sup>g</sup>	14.71 ± 0.04 <sup>f</sup>	16.46 ± 0.05 <sup>e</sup>	18.67 ± 0.01 <sup>d</sup>	20.67 ± 0.02 <sup>c</sup>	2.77 ± 0.08 <sup>b</sup>	24.94 ± 0.14 <sup>a</sup>
<b>Fiber</b>	13.93 ± 0.12 <sup>e</sup>	16.96 ± 0.09 <sup>d</sup>	17.62 ± 0.23 <sup>c</sup>	19.05 ± 0.21 <sup>b</sup>	18.67 ± 0.01 <sup>b</sup>	19.90 ± 0.12 <sup>a</sup>	20.41 ± 0.12 <sup>a</sup>	18.76 ± 0.18 <sup>b</sup>

### 3.3. Microbiological Characteristics of Larvae Flour and Local Flours

In the formulation, the microbiological quality of the larvae meal, fish meal, corn flour, soybean flour and low-grade rice flour was assessed. Germs such as *Vibrio*, *Pseudomonas*, *E. coli*, Anaerobic Sulfite-Reducing (ASR) were found but *Salmonella* was absent from the various flours used in the formulation. Fecal Streptococci were only counted in the larval flour with a load of 8.6, 10<sup>2</sup> CFU/g. Yeasts and molds were only detected in the corn flour with an average load of 1.6, 10<sup>2</sup> CFU/g. Staphylococci and *Aeromonas* were only detected in low-grade rice flour with loads of 2.6, 10<sup>2</sup> CFU/g and 2.6, 10<sup>3</sup> CFU/g respectively (**Table 4**)

**Table 4.** Level of contamination of the different flours used for formulation.

Germs (CFU/g)	Flour						Criteria
	Corn	Rice	Larva	Fish	Soybean		
<b>Fecal Streptococci</b>	<1 <sup>b</sup>	<1 <sup>b</sup>	(8.6 ± 0.1) 10 <sup>2 a</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>	10 <sup>4</sup> à 10 <sup>5</sup>	
<b><i>Staphylococcus</i></b>	<1 <sup>b</sup>	(2.6 ± 0.1) 10 <sup>2 a</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>	10 <sup>3</sup> à 10 <sup>4</sup>	
<b><i>Aeromonas</i></b>	<1 <sup>b</sup>	(2.6 ± 0.0) 10 <sup>3 a</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>	-	
<b><i>Pseudomonas</i></b>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	-	
<b><i>Vibrio</i></b>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	10 <sup>6</sup>	
<b><i>E. coli</i></b>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	10 à 10 <sup>2</sup>	
<b>ASR</b>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	<1 <sup>a</sup>	10 <sup>2</sup> à 10 <sup>3</sup>	
<b>YM</b>	(1.6 ± 0.1) 10 <sup>2 a</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>	<1 <sup>b</sup>	10 <sup>2</sup> à 10 <sup>3</sup>	
<b><i>Salmonella</i></b>	Absence	Absence	Absence	Absence	Absence	Absence	

In the same line and for the same types of microorganisms, the values bearing

different letters are significantly different at the threshold  $\alpha = 0.05$ .

CFU/g Colony-forming Unit per g of the different flours.

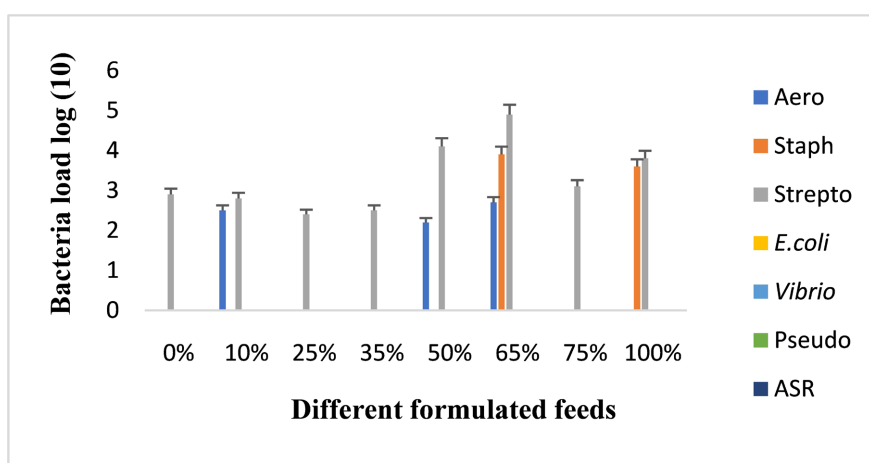
ASR Anaerobic Sulfite-Reducing.

YM Yeast and Mold.

### 3.4. Microbiological Characteristics of Formulated Feeds

#### 3.4.1. Bacterial Flora Contamination

Indicative bacteria of fecal contamination, such as *E. coli* and Anaerobic Sulfite-Reducing were not detected in all feeds formulated with *Hermetia illucens* larvae. Fecal Streptococci were detected in all formulated feeds, with loads ranging from 2.4 to 4.9 log<sub>10</sub> CFU/g. *Vibrio*, *Pseudomonas* and *Salmonella* were not detected in any of the formulated feeds. *Aeromonas* was detected in feed formulated at 10%, 50% and 65%, with loads ranging from 2.2 to 2.7 log<sub>10</sub> CFU/g. *Staphylococcus* were detected in the 65% and 100% formulated foods, with loads ranging from 3.6 to 3.9 log<sub>10</sub> CFU/g. The most contaminated feed was the 65% formulation, with the presence of *Aeromonas*, *Staphylococcus* and *Streptococcus* (Figure 1).



Aero: *Aeromonas*; Staph: *Staphylococcus*; Strepto: *Streptococcus*; Pseudo: *Pseudomonas*; ASR: Anaerobic Sulfite-Reducing.

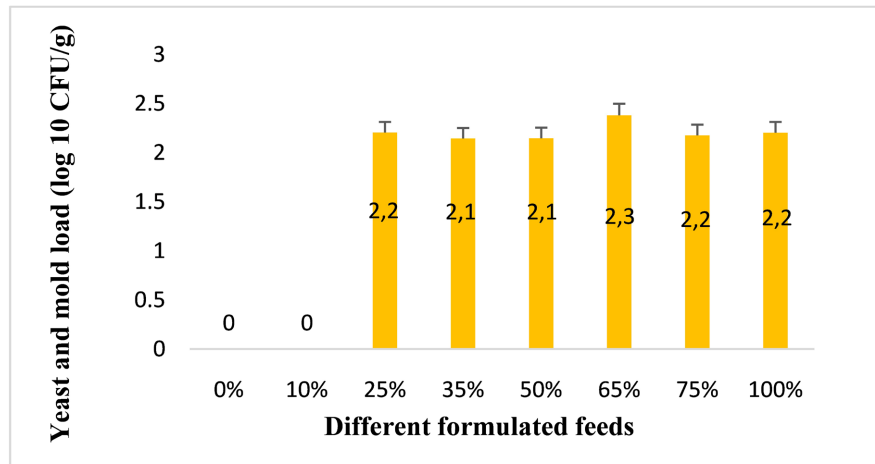
**Figure 1.** Level of bacterial contamination of various formulated feeds.

#### 3.4.2. Fungal Contamination

Yeasts and molds were detected in feed formulated at 25%, 35%, 50%, 65%, 75% and 100%, with loads ranging from 2.1 to 2.3 log<sub>10</sub> CFU/g. In the 0% and 10% formulated feeds, no yeast and mold load were detected (Figure 2).

### 3.5. Microbiological Quality of Formulated Feeds

In terms of the microbiological quality of the formulated feeds, the 0% and 10% feeds were of satisfactory microbiological quality, while the 25%, 35%, 50%, 65%, 75% and 100% feeds were of acceptable microbiological quality according to ISO 11133: 2014 (Table 5).



**Figure 2.** Yeast and mold contamination levels in various formulated feeds.

**Table 5.** Microbial quality of formulated feeds (CFU/g).

Germs	Formulated Feeds								Criteria	
	Feed 0%	Feed 10%	Feed 25%	Feed 35%	Feed 50%	Feed 65%	Feed 75%	Feed 100%	m	M
<i>E. coli</i>	<1	<1	<1	<1	<1	<1	<1	<1	10	10 <sup>2</sup>
Strep f	8.3, 10 <sup>2</sup>	5.5, 10 <sup>2</sup>	2.3, 10 <sup>2</sup>	3.2, 10 <sup>2</sup>	1.1, 10 <sup>4</sup>	9.5, 10 <sup>4</sup>	1.1, 10 <sup>3</sup>	5.1, 10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>5</sup>
ASR	<1	<1	<1	<1	<1	<1	<1	<1	10 <sup>2</sup>	10 <sup>3</sup>
<i>Vibrio</i>	<1	<1	<1	<1	<1	<1	<1	<1		10 <sup>6</sup>
Pseudo	<1	<1	<1	<1	<1	<1	<1	<1	-	-
Staph	<1	<1	<1	<1	<1	7.4, 10 <sup>3</sup>	<1	4, 10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>
Aero	<1	3.4, 10 <sup>2</sup>	<1	<1	1.7, 10 <sup>2</sup>	4.3, 10 <sup>3</sup>	<1	<1		10 <sup>4</sup>
YM	<1	<1	1.6, 10 <sup>2</sup>	1.3, 10 <sup>2</sup>	1.3, 10 <sup>2</sup>	2.4, 10 <sup>2</sup>	1.5, 10 <sup>2</sup>	1.5, 10 <sup>2</sup>	10 <sup>2</sup>	10 <sup>3</sup>
Salm	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs		Abs
	S	S	A	A	A	A	A	A		

Strep f: Fecal Streptococci; Staph: *Staphylococcus*; ASR: Anaerobic Sulfite-Reducing; Pseudo: *Pseudomonas*; Salm: *Salmonella*; YM: Yeasts and Molds; Aero: *Aeromonas*; Abs: Absence; A: Acceptable; S: Satisfactory.

### 4. Discussion

Incorporating black soldier fly larvae meal into fish feed composed of fish, corn, low-grade rice and soybean meal had an influence on the biochemical properties. The low humidity recorded could be due to the prior drying of the matrices used. FAO [15] recommends a moisture content of less than 10% to preserve flour-based products for reasonable periods. The addition of larvae meal resulted in an increase in fiber content ranging from (13.93 to 20.41%) and a decrease in ash content ranging from (20.27 to 8.15%). The increase was also reported by Brou *et al.* [16] in

their work on the effects of extruded feeds containing rice bran and wheat bran on the growth performance of tilapia *Oreochromis niloticus* reared in pens. The authors' work revealed that diets with low fiber content gave better results than those with high fiber content. Another study by Lazard [17] indicated that a low-fiber diet was conducive to good fish growth. Regarding ash content, Lazard [17] recommended an ash content of less than 10% for good fish growth. However, Bamba *et al.* [18] found good growth performance with an ash content of 15.38%. In general, feeds formulated at 0% and 10% give low fiber and high ash content compared to other formulated feeds. As far as lipids are concerned, the content increases significantly with the rate of incorporation of larvae meal. According to Rivière [19], an increase in lipid content, in reasonable proportions in the feed, can lead to an economy in the use of protein in fish, without altering the quality of the feed. MSN larvae have a higher lipid content than fish meal. This could therefore explain the higher lipid content in feeds formulated at 100%, 75%, 65% and 50%.

The results of microbiological analyses carried out on flour and formulated feed revealed the presence of certain germs. The loads of yeasts and molds, as well as those of fecal Streptococci, *Aeromonas* and *Staphylococcus* recorded in flours and formulated feeds based on maize, larvae and poor rice are below the microbiological standard, which is 103 CFU/g, 105 CFU/g and 104 CFU/g respectively [20]. This low load could be explained by the low water content due to the prior drying of the matrices used [21]. Indeed, several authors have shown that a significant reduction in water content can reduce the total flora in flours in Côte d'Ivoire [22]. The presence of faecal Streptococci detected in all formulated feeds is mainly due to their origin in BSF larvae meal. According to Klunder *et al.* [23], the presence of a wide range of micro-organisms in the insect digestive tract could be a high source of contamination, as larvae are processed whole without evisceration due to their small size. Staphylococci and *Aeromonas* were found in most of the formulated feeds (10%, 50%, 65% and 100%). This contamination could therefore be due to the addition of low-quality rice flour previously contaminated with these germs. Yeasts and molds were observed in the formulated feeds (25%, 35%, 50%, 65%, 75% and 100%). Their presence in these feeds could be due to the corn flour. The fungal and bacteriological loads detected were below the microbiological criteria applicable to animal feed [18].

Evaluation of the overall microbiological quality showed that the highest loads were detected in feeds formulated at 65% and the lowest loads in formulated feeds at 0% and 10%. As Cabarkapa *et al.* [24] point out, feed can become contaminated during processing, storage or transport. Feed ingredients must be safe and suitable for storage. A single hazardous component can compromise the quality of the compound feed. High-quality raw materials can lead to the production of high-quality feed.

## 5. Conclusion

This study determined the nutritive and microbiological quality of fish feed

formulated from local flours enriched with *Hermetia illucens* larvae. Several germs were found in the flours used for formulation as well as in the formulated feeds, but at loads below the microbiological criteria applicable to animal feeds. The microbiological quality of these eight formulated feeds was found to be acceptable. In general, all eight feeds were of good nutritive quality. However, the 0% and 10% formulated feeds showed the best nutritive and microbiological characteristics. The results obtained suggest that flours enriched with *Hermetia illucens* larvae could be used in fish feed.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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