

# Microbial assessment of *Clarias gariepinus* fillets fed defatted and non-defatted sundried black soldier fly larvae meal

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**ABSTRACT:** Aquaculture production in Nigeria is hindered by the dependence on fishmeal as the main protein source in fish feed. Consequently, there is a need to explore alternative protein sources that is cost-effective, sustainable and pose no risk to consumers. This study evaluated the microbial loads and isolates in *Clarias gariepinus* fillets fed defatted (DF) and non-defatted (NDF) sundried black soldier fly larvae meal (BSFLM) diets over a period of 21 storage days with assessments at day 0, 7th, 14th and 21st, after 168 days of feeding trials. Fishmeal was replaced with DF and NDF sundried BSFLM at varying inclusion levels of 0%, 25%, 50%, 75% and 100% in the diets of *Clarias gariepinus*, which were fed for a period of 168 days. Following the feeding trial, the fish were saline-rinsed, filleted, and sealed in double polyethylene bags. The fillets underwent boiling for 20 minutes, after which the fish were foil-wrapped, grouped in ziplock bags, labelled and stored at 4°C for 21 days in a chest freezer. Microbial analysis was estimated for each labelled ziplock bag sample at days 0, 7th, 14th, and 21st of the storage period, assessing total bacterial count (TBC), total coliform count (TCC), mould yeast, and microbe isolates. The results obtained showed that at day zero of storage, the highest TBC ( $8.0 \times 10^5$  CFU/g) was observed in fillets fed 75% defatted BSFLM, with *Bacillus* and *Candida* spp. dominating the isolates. At seven days of storage, the TBC ranged from  $1.02 \times 10^2$  CFU/g (0% defatted BSFLM) to  $9.6 \times 10^5$  CFU/g (75% defatted BSFLM), with *Staphylococcus*, *Bacillus*, and *Candida* spp. being the most persistent isolates. At the twenty-first day of storage, TBC in fish fillets fed 0% and 25% defatted BSFLM were more than 300 colony counts, which were recorded as too numerous to count because they exceeded permissible limits for consumption, and a broader spectrum of microorganisms, including *Staphylococcus* spp., *Bacillus*, *Aspergillus* spp., and *Klebsiella* spp., were isolated. This study concluded that catfish fillets fed defatted and non-defatted sundried BSFLM diets should be consumed within a shorter storage period due to the potential microbial risks associated with prolonged storage.

**Keywords:** *Candida* spp., fish health, fishmeal substitutes, microorganisms, permissible limits, *Staphylococcus* spp.

## INTRODUCTION

Fishmeal (FM) plays an important role in the fish feed industry, owing to its high nutritional value that aligns with the needs of many cultivated fish species, making it a key factor in their success. According to Gebremichael *et al.* (2023), the global fishmeal (FM) supply has dwindled

rapidly due to declining capture fisheries, an increase in human consumption, and competition from the animal feed industry, thereby leading to soaring feed costs. This reliance on FM as the main protein source in fish feed has hindered aquaculture production in Nigeria (Shati *et al.*,

2022). Consequently, fish nutritionists are seeking alternative protein sources that are affordable, high-quality, non-competitive, and safe for both primary and secondary consumers.

The Black Soldier Fly (BSF), (*Hermetia illucens*), is a promising insect species with significant potential (Rehman *et al.*, 2023; da-Silva *et al.*, 2024). Black Soldier Fly larvae (BSFL) have been gaining attention for their potential in animal feed production due to their ability to utilise cheap organic waste, fast-growing rate, and disease tolerance (Rehman *et al.*, 2023; Iyapo *et al.*, 2025). Studies have shown that locally produced BSFL have a suitable nutritional composition for commercial fish feed formulation, offering a viable alternative to conventional fishmeal (Rehman *et al.*, 2023; da-Silva *et al.*, 2024; Iyapo *et al.*, 2025).

Fish quality has become a priority for consumers and the aquaculture industry due to its direct impact on human health and nutrition (Adebiyi *et al.*, 2023). Fish freshness is a key indicator of its palatability, safety, market value, and consumer acceptability, as fish quality declines rapidly during storage due to enzymatic and oxidative rancidity, resulting in significant value loss. Post-mortem deterioration, caused by enzymatic activity and lipid oxidation, results in muscle weakening and tissue breakdown, creating opportunities for microbial invasion of the fish fillet (Chaklader *et al.*, 2023).

Microbial activities are the primary cause of spoilage in fresh and lightly preserved fish, which generate off odours and flavours through metabolic processes (Gerges *et al.*, 2016). The shelf life of fishery products is typically affected by microbial growth, which is largely determined by numerous factors such as storage period, temperature and humidity. The total microbial load in fishery products is used to assess the quality and safety of the products (Milijašević *et al.*, 2024). Research by Gerges *et al.* (2016) established that fresh fish should not exceed  $\log_{10}^7$  CFU/g of total bacterial count, while chilled fish should not exceed  $\log_{10}^6$  CFU/g of total viable count.

During storage, changes in microbial activity on fish fillets trigger various chemical reactions that can indicate microbial loads and types. Consumption of spoiled fish poses significant health risks to consumers (Moutinho *et al.*, 2021). Studies have explored the effects of replacing fishmeal with Black Soldier Fly larvae meal (BSFLM) on fish development and health benefits (Agbohessou *et al.*, 2020; Abdel-Tawwab *et al.*, 2020; Moutinho *et al.*, 2021), but limited information was documented on microbial loads and counts of fish fed BSFLM.

Catfish is the most widely farmed and consumed fish in Africa (Chen *et al.*, 2023). However, the industry's heavy reliance on fishmeal as a primary protein source has led to fishmeal price increases. Therefore, a sustainable and safe alternative protein source that will not pose a threat to consumers is needed. Black soldier fly larvae provide a sustainable solution for waste management and a rich source of protein and nutrients (Gul *et al.*, 2024; Muin *et*

*al.*, 2024). The purpose of this experiment is to assess the microbial loads and counts of *Clarias gariepinus* fillets fed defatted and non-defatted sundried black soldier fly larvae meal diets as an alternative to fishmeal diets for 168 days.

## MATERIALS AND METHODS

### Experimental site

The feeding trial was conducted at Aquaculture and Fisheries Management, Department of Renewable Natural Resources, University of Ibadan; fillet preparation was conducted at the Animal Science Department, and microbial analysis was done at the Biochemistry Department, University of Ibadan, Ibadan, Nigeria.

### Experimental procedure

The experimental fish were purchased from a reputable fish farm at Ibadan, Oyo State, while black soldier fly larvae were obtained from the BSFL culturing unit at the Aquaculture and Fisheries Department, University of Ibadan, Ibadan, Nigeria. The larvae were processed and incorporated into feed formulations with other ingredients.

### Experimental fish processing

After 168 days of feeding trial, the fish were rinsed in 0.1% salt solution, cut into pieces (50 g each), sealed in double polyethylene bags and steam-cooked for 20 minutes. The cooked fish were cooled, wrapped in foil paper and coded with 3 digits. The fillets were grouped and packed in a Ziplock bag, labelled, and stored inside the departmental freezer. Samples were analysed at intervals every seven days for 21 storage period for microbial load and isolates.

### Microbial assessment

The microbiological quality and safety of *C. gariepinus* fillets fed defatted and non-defatted sundried black soldier fly larvae meal diets as an alternative to fishmeal diets over a period of 168 days of feeding trials were evaluated based on Total Bacterial Count (TBC), Total Coliform Count (TCC), moulds, yeast, and microbes isolates. This involved homogenising 10 g of fish fillet with buffered peptone water, followed by serial dilution and pour plating on specific agar media, including Tryptic Soy Agar, Plate Count Agar, MacConkey Agar, Mannitol Salt Agar, and Salmonella Shigella Agar. After incubation at 37°C for 48 hours, colonies were counted and expressed as  $\log_{10}$  CFU/g. Microbes Isolate were identified and characterised through morphological, cultural, and biochemical tests, including motility, catalase, coagulase, oxidase, and other

**Table 1.** Microbial load (cfu/g) of *C. gariepinus* fillets fed defatted and non-defatted sundried black soldier fly larvae meal diets at day Zero.

Treatments (%)	TBC (Cfu/g)	TCC (Cfu/g)	Mould (Cfu/g)	Yeast (Cfu/g)
D1	$8.0 \times 10^6$	0	0	0
D2	$2.8 \times 10^5$	0	$2.0 \times 10^2$	$1.0 \times 10^2$
D3	0	0	0	$12.0 \times 10^1$
D4	0	0	0	$16.0 \times 10^1$
D5	0	0	0	$2.0 \times 10^2$
ND1	$7.0 \times 10^6$	0	0	0
ND2	0	0	0	$5.0 \times 10^2$
ND3	$1.3 \times 10^5$	0	0	$2.2 \times 10^1$
ND4	TNTC	0	0	$24.0 \times 10^1$
ND5	$2.6 \times 10^4$	0	0	$1.0 \times 10^2$

TBC = total bacteria count; TCC= total coliform count; D1= Diet with 0% defatted black soldier fly larvae meal : 100% fishmeal; D2 = Diet with 25% defatted black soldier fly larvae meal : 75% fishmeal; D3 = Diet with 50% defatted black soldier fly larvae meal : 50% fishmeal; D4 = Diet with 75% defatted black soldier fly larvae meal : 25% fishmeal, D5 = Diet with 100% defatted black soldier fly larvae meal : 0% fishmeal; ND1 = Diet with 0% non-defatted black soldier fly larvae meal : 100% fishmeal; ND2 = Diet with 25% non-defatted black soldier fly larvae meal : 75% fishmeal; ND3 = Diet with 50% non-defatted black soldier fly larvae meal : 50% fishmeal; ND4 = Diet with 75% non-defatted black soldier fly larvae meal : 25% fishmeal; ND5 = Diet with 100% non-defatted black soldier fly larvae meal : 0% fishmeal; TNTC = Too numerous to count.

standard assays for microbial identification (Perera *et al.*, 2024).

### Statistical analysis

The data generated for total bacterial count (TBC), total coliform count (TCC), moulds and yeast for *C. gariepinus* fillets fed with different dietary formulations were transformed from CFU/g to  $\log_{10}$  CFU/g.

## RESULTS

The results on the composition of defatted and non-defatted sundried BSFLM and growth performance on *C. gariepinus* fed have been published in reputable journals. This study analysed the microbial counts, loads and isolates of *C. gariepinus* fillets fed defatted and non-defatted sundried BSFLM diets during 21 days storage period, after 168 days of feeding trials. On day zero (0), the highest total bacterial count (TBC) ( $3.0 \times 10^8$  CFU/g) was recorded in fish fillet fed ND4 (75% non-defatted sundried BSFLM) diets, followed by fish fillet fed D1 (0% defatted sundried BSFLM) ( $8.0 \times 10^6$  CFU/g), while the lowest TBC ( $1.3 \times 10^5$  CFU/g) was recorded in fish fillet fed ND3 (50% non-defatted sundried BSFLM) diet. Notably, TBC was undetectable in fish fillets fed D3 (50% defatted sundried BSFLM), D4 (75% defatted sundried BSFLM), D5 (100% defatted sundried BSFLM), and ND2 (25% non-defatted sundried BSFLM) diets. Total coliform count (TCC) was not detected in any of the fish fillets, regardless of the diet.

Mould was also absent in most treatments at day zero of storage, except in fish fillet fed D2 (25% defatted sundried BSFLM) diet, which recorded ( $2.0 \times 10^2$  CFU/g). Yeast

counts varied, with the highest level found in fish fillet fed ND4 (75% non-defatted sundried BSFLM) diet reported having  $24.0 \times 10^1$  CFU/g, followed by ( $16.0 \times 10^1$  CFU/g) fish fillet fed D4 (75% defatted sundried BSFLM) diet, and the lowest yeast value of  $1.0 \times 10^2$  CFU/g recorded in fish fillets fed D2 (25% defatted sundried BSFLM) and ND5 (100% non-defatted sundried BSFLM) diets. Notably, yeast was not detected in fillets fed D1 (0% defatted sundried BSFLM) and ND1 (0% non-defatted sundried BSFLM) diets (Table 1). The microbial isolates revealed a prevalence of *Bacillus spp.* and *Saccharomyces spp.*, indicating that these bacteria and fungi (yeast) were inherently present in the fish fillets across both defatted and non-defatted BSFLM diet groups (Table 2).

At the seventh day of storage, microbial loads increased in fish fillets fed both defatted and non-defatted sundried BSFLM diets, with fish fillets fed the ND3 (75% non-defatted sundried BSFLM) diet having the highest TBC of  $8.8 \times 10^5$  CFU/g and fish fillets fed the ND2 (25% non-defatted sundried BSFLM) diet having the lowest value of  $1.16 \times 10^2$  CFU/g total bacterial counts. Yeast and mould were present in some samples, while *Staphylococcus spp.* was consistently detected, indicating their pre-existing presence. The increasing values of microbial growth likely reflect the nutrient-rich environment provided by the diets (Table 3). The isolated microbes from the fish fillets on the seventh day of storage included *Staphylococcus spp.*, *Botrytis spp.*, *Candida spp.*, and *Bacillus spp.*, suggesting favourable environmental conditions for their growth (Table 4).

At the fourteenth day of storage, the microbial profiles had shifted, with some diets showing lower total bacterial counts (TBC). Notably, fish fillets fed ND4 (75% non-defatted sundried BSFLM) diet had relatively low ( $1.16 \times 10^4$  CFU/g) TBC, while those fish fillets fed ND5 (100% non-defatted sundried BSFLM) diet had the highest TBC

**Table 2.** Isolated organisms of *C. gariepinus* filets fed defatted and non-defatted sundried black soldier fly larvae meal diet at day zero.

Treatments (%)	Bacteria	Mould	Yeast
D1	<i>Bacillus spp</i>	-	-
D2	<i>Staphylococcus spp</i>	<i>Fusarium spp</i>	<i>Candida spp</i>
D3	-	-	<i>Saccharomyces spp</i>
D4	-	-	<i>Saccharomyces spp, Candida spp</i>
D5	-	-	<i>Saccharomyces spp, Candida spp</i>
ND1	<i>Staphylococcus spp</i>	-	-
ND2	-	-	<i>Saccharomyces spp</i>
ND3	<i>Bacillus spp</i>	-	<i>Saccharomyces spp</i>
ND4	<i>Bacillus spp</i>	-	<i>Saccharomyces spp</i>
ND5	<i>Bacillus spp</i>	-	<i>Saccharomyces spp, Candida spp</i>

Legend: D1= Diet with 0% defatted black soldier fly larvae meal; D2 = Diet with 25% defatted black soldier fly larvae meal; D3 = Diet with 50% defatted black soldier fly larvae meal; D4 = Diet with 75% defatted black soldier fly larvae meal, D5 = Diet with 100% defatted black soldier fly larvae meal; ND1 = Diet with 0% non-defatted black soldier fly larvae meal; ND2 = Diet with 25% non-defatted black soldier fly larvae meal; ND3 = Diet with 50% non-defatted black soldier fly larvae meal; ND4 = Diet with 75% non-defatted black soldier fly larvae meal; ND5 = Diet with 100% non-defatted black soldier fly larvae meal.

**Table 3.** Microbial load (cfu/ml) of *Clarias gariepinus* filets defatted and non-defatted sundried black soldier fly larvae meal diets at day seventh.

Treatments (%)	TBC (Cfu/g)	Staphylococcus (Cfu/g)	TCC (Cfu/g)	Mould (Cfu/g)	Yeast (Cfu/g)
D1	1.32 x 10 <sup>2</sup>	9.8 x 10 <sup>2</sup>	0	2.0 x 10 <sup>2</sup>	6.0 x 10 <sup>2</sup>
D2	6.0 x 10 <sup>5</sup>	8.6 x 10 <sup>2</sup>	0	3.0 x 10 <sup>2</sup>	1.3 x 10 <sup>2</sup>
D3	1.8 x 10 <sup>2</sup>	26.0 x 10 <sup>2</sup>	0	3.0 x 10 <sup>2</sup>	0
D4	3.8 x 10 <sup>4</sup>	6.2 x 10 <sup>2</sup>	0	1.0 x 10 <sup>2</sup>	26.0 x 10 <sup>2</sup>
D5	1.7 x 10 <sup>5</sup>	14.0 x 10 <sup>2</sup>	0	0	26.8 x 10 <sup>2</sup>
ND1	4.8 x 10 <sup>5</sup>	14.8 x 10 <sup>2</sup>	0	0	3.0 x 10 <sup>2</sup>
ND2	1.16 x 10 <sup>2</sup>	18.0 x 10 <sup>2</sup>	0	0	0
ND3	8.8 x 10 <sup>5</sup>	12.0 x 10 <sup>2</sup>	0	0	6.0 x 10 <sup>1</sup>
ND4	6.8 x 10 <sup>5</sup>	5.4 x 10 <sup>2</sup>	0	2.0 x 10 <sup>2</sup>	7.2 x 10 <sup>2</sup>
ND5	1.28 x 10 <sup>2</sup>	11.0 x 10 <sup>2</sup>	0	2.0 x 10 <sup>2</sup>	3.0 x 10 <sup>1</sup>

TBC= total bacteria count; TCC= total coliform count; D1= Diet with 0% defatted black soldier fly larvae meal; D2 = Diet with 25% defatted black soldier fly larvae meal; D3 = Diet with 50% defatted black soldier fly larvae meal; D4 = Diet with 75% defatted black soldier fly larvae meal, D5 = Diet with 100% defatted black soldier fly larvae meal; ND1 = Diet with 0% non-defatted black soldier fly larvae meal; ND2 = Diet with 25% non-defatted black soldier fly larvae meal; ND3 = Diet with 50% non-defatted black soldier fly larvae meal; ND4 = Diet with 75% non-defatted black soldier fly larvae meal; ND5 = Diet with 100% non-defatted black soldier fly larvae meal.

of 7.6 x 10<sup>5</sup> CFU/g. This shift may indicate stabilisation or adaptation of the microbial community to the dietary components. Total coliform count (TCC) was only detected in fish fillet fed ND5 (100% non-defatted sundried BSFLM) diet (3.6 x 10<sup>2</sup> CFU/g), suggesting a potential diet-specific effect. Moulds were only detected in fish fillets fed D3 (50% defatted sundried BSFLM), ND1 (0% non-defatted sundried BSFLM) and ND2 (25% non-defatted sundried BSFLM) diets, while yeast was present in all the treatments (Table 5). *Staphylococcus spp.*, *Bacillus spp.*, and *Candida spp.* were frequently detected on fish fillets fed both defatted and non-defatted sundried BSFLM diets. Additionally, *Salmonella spp.* and *Klebsiella spp.* were found on fillets fed the ND5 diet, while *E. coli* was present

on fish fillet fed ND1 (0% non-defatted sundried BSFLM) diet. This suggests that the fish fillets provided a conducive environment for the growth of these newly formed microorganisms (Table 6).

At the twenty-first day of storage, microbial counts revealed a complex dynamic. *Staphylococcus spp.* and other bacteria were abundant in fish fillets fed ND1 (0% non-defatted sundried BSFLM) and ND2 (25% non-defatted sundried BSFLM) diets. Notably, fish fillet fed the ND1 (0% non-defatted sundried BSFLM) diet showed the highest total coliform count (TCC) of 8.5 x 10<sup>8</sup> CFU/g, which was recorded as too numerous to count (TNTC) because it exceeded permissible limits for consumption, indicating a significant presence of coliforms. In addition,

**Table 4.** Isolated organisms of *Clarias gariepinus* fillets fed defatted and non-defatted sundried black soldier fly larvae meal diets at Day seventh.

Treatments (%)	Bacteria	Mould	Yeast
D1	<i>Staph spp</i>	<i>Botrytis spp</i>	<i>Saccharomyces spp</i>
D2	<i>Bacillus spp, Staph spp</i>	<i>Alternaria spp, Botrytis spp</i>	<i>Candida spp</i>
D3	<i>Bacillus spp, Staph spp</i>	<i>Mucor spp, Botrytis spp, Fusarium spp, Aspergillus spp</i>	-
D4	<i>Bacillus spp, Staph spp</i>	<i>Mucor spp</i>	<i>Candida spp</i>
D5	<i>Bacillus spp, Staph spp</i>	-	<i>Candida spp</i>
ND1	<i>Bacillus spp, Staph spp</i>	-	<i>Candida spp</i>
ND2	<i>Bacillus spp, Staph spp</i>	-	-
ND3	<i>Bacillus spp, Staph spp</i>	-	<i>Candida spp</i>
ND4	<i>Bacillus spp, Staph spp</i>	<i>Fusarium spp, Aspergillus spp</i>	<i>Candida spp</i>
ND5	<i>Bacillus spp, Staph spp</i>	<i>Mucor spp, Botrytis spp</i>	<i>Candida spp</i>

**Legend:** D1= Diet with 0% defatted black soldier fly larvae meal; D2 = Diet with 25% defatted black soldier fly larvae meal; D3 = Diet with 50% defatted black soldier fly larvae meal; D4 = Diet with 75% defatted black soldier fly larvae meal, D5 = Diet with 100% defatted black soldier fly larvae meal; ND1 = Diet with 0% non-defatted black soldier fly larvae meal; ND2 = Diet with 25% non-defatted black soldier fly larvae meal; ND3 = Diet with 50% non-defatted black soldier fly larvae meal; ND4 = Diet with 75% non-defatted black soldier fly larvae meal; ND5 = Diet with 100% non-defatted black soldier fly larvae meal.

**Table 5.** Microbial load (cfu/ml) of *Clarias gariepinus* fillets defatted and non-defatted sundried black soldier fly larvae meal diets at day fourteenth.

Treatments (%)	TBC (Cfu/g)	Staphylococcus (Cfu/g)	TCC (Cfu/g)	Mould(Cfu/g)	Yeast (Cfu/g)
D1	2.0 x 10 <sup>4</sup>	0	0	0	3.6 x 10 <sup>2</sup>
D2	2.1 x 10 <sup>5</sup>	0	0	0	1.4 x 10 <sup>2</sup>
D3	3.4 x 10 <sup>5</sup>	6.0 x 10 <sup>2</sup>	0	1.0 x 10 <sup>1</sup>	8.0 x 10 <sup>2</sup>
D4	4.4 x 10 <sup>5</sup>	0	0	0	1.1 x 10 <sup>2</sup>
D5	1.2 x 10 <sup>5</sup>	0	0	0	7.0 x 10 <sup>2</sup>
ND1	2.0 x 10 <sup>4</sup>	7.2 x 10 <sup>2</sup>	0	2.0 x 10 <sup>2</sup>	1.8 x 10 <sup>2</sup>
ND2	1.8 x 10 <sup>4</sup>	8.6 x 10 <sup>2</sup>	0	8.0 x 10 <sup>2</sup>	1.0 x 10 <sup>1</sup>
ND3	1.7 x 10 <sup>4</sup>	0	0	0	3.2 x 10 <sup>2</sup>
ND4	1.2 x 10 <sup>4</sup>	0	0	0	1.4 x 10 <sup>2</sup>
ND5	7.6 x 10 <sup>5</sup>	0	3.6 x 10 <sup>2</sup>	0	4.0 x 10 <sup>2</sup>

**Legend:** TBC= total bacteria count; TCC= total coliform count; D1= Diet with 0% defatted black soldier fly larvae meal; D2 = Diet with 25% defatted black soldier fly larvae meal; D3 = Diet with 50% defatted black soldier fly larvae meal; D4 = Diet with 75% defatted black soldier fly larvae meal, D5 = Diet with 100% defatted black soldier fly larvae meal; ND1 = Diet with 0% non-defatted black soldier fly larvae meal; ND2 = Diet with 25% non-defatted black soldier fly larvae meal; ND3 = Diet with 50% non-defatted black soldier fly larvae meal; ND4 = Diet with 75% non-defatted black soldier fly larvae meal; ND5 = Diet with 100% non-defatted black soldier fly larvae meal.

yeast was recorded TNTC in fish fillets fed ND1 (0% non-defatted sundried BSFLM) and ND2 (25% non-defatted sundried BSFLM) diets because the value (8.6 x10<sup>8</sup> CFU/g) recorded is above the permissible limit for human consumption (Table 7). The detection of *Aspergillus spp.* and *Klebsiella spp.* indicates a varied microbial population, highlighting the diets' potential to facilitate diverse microbial growth (Table 8).

## DISCUSSION

Microbial activity is a major cause of spoilage in fresh and

lightly preserved seafood (Gerges *et al.*, 2016). Total microbial loads and counts are often used to assess the safety and quality of seafood products (Milijašević *et al.*, 2024). According to Gerges *et al.* (2016), the acceptable limit for total bacterial count and total coliform count in fresh fish is 7 log<sub>10</sub> CFU/g and 6 log<sub>10</sub> CFU/g for chilled fish. Additionally, Gerges *et al.* (2016) also noted that a product is considered spoiled or near spoilage when the aerobic plate counts reach 15.0 x 10<sup>6</sup> CFU/g. The aerobic plate counts throughout the 21days storage period (days 0, 7, 14, and 21) in this research were below 15.0 x 10<sup>6</sup> CFU/g, indicating that the fish fillets fed defatted and non-defatted sundried black soldier fly larvae meal diets remained fit for

**Table 6.** Isolated organisms of *Clarias gariepinus* fillets fed defatted and non-defatted sundried black soldier fly larvae meal diets at day fourteenth.

Treatments (%)	Bacterial	Mould	Yeast
D1	<i>Bacillus spp</i>	-	<i>Candida spp</i>
D2	<i>Staph spp</i>	-	<i>Candida spp</i>
D3	<i>Bacillus</i>	<i>Botrytis spp</i>	<i>Candida spp</i>
D4	<i>Staph spp</i>	-	<i>Candida spp</i>
D5	<i>Bacillus spp, Staph spp</i>	-	<i>Candida spp, saccharomyces spp</i>
ND1	<i>Staph spp, Bacillus, E.coli,</i>	<i>Botrytis spp</i>	<i>Candida spp, saccharomyces spp</i>
ND2	<i>Staph spp, Bacillus</i>	<i>Botrytis spp</i>	<i>Candida spp,</i>
ND3	<i>Staph spp</i>	-	<i>Candida spp</i>
ND4	<i>Staph spp</i>	-	<i>Candida spp</i>
ND5	<i>Staph spp, Staph spp, Salmonella, Kleb</i>	-	<i>Candida spp</i>

**Legend:** D1= Diet with 0% defatted black soldier fly larvae meal; D2 = Diet with 25% defatted black soldier fly larvae meal; D3 = Diet with 50% defatted black soldier fly larvae meal; D4 = Diet with 75% defatted black soldier fly larvae meal, D5 = Diet with 100% defatted black soldier fly larvae meal; ND1 = Diet with 0% non-defatted black soldier fly larvae meal; ND2 = Diet with 25% non-defatted black soldier fly larvae meal; ND3 = Diet with 50% non-defatted black soldier fly larvae meal; ND4 = Diet with 75% non-defatted black soldier fly larvae meal; ND5 = Diet with 100% non-defatted black soldier fly larvae meal.

**Table 7.** Microbial load (cfu/ml) of *Clarias gariepinus* fillets fed defatted and non-defatted sundried black soldier fly larvae meal diets at day twenty-first.

Treatments (%)	TBC (Cfu/g)	Staphylococcus (Cfu/g)	TCC (Cfu/g)	Mould (Cfu/g)	Yeast (Cfu/g)
D1	5.2 x 10 <sup>5</sup>	3.0 x 10 <sup>1</sup>	2.0 x 10 <sup>2</sup>	2.0 x 10 <sup>2</sup>	8.0 x 10 <sup>2</sup>
D2	7.6 x 10 <sup>5</sup>	0	0	0	5.0 x 10 <sup>2</sup>
D3	4.0 x 10 <sup>5</sup>	5.0 x 10 <sup>2</sup>	2.0 x 10 <sup>2</sup>	0	1.5 x 10 <sup>2</sup>
D4	1.2 x 10 <sup>4</sup>	9.0 x 10 <sup>2</sup>	6.0 x 10 <sup>2</sup>	0	1.6 x 10 <sup>2</sup>
D5	8.2 x 10 <sup>5</sup>	4.0 x 10 <sup>2</sup>	4.0 x 10 <sup>2</sup>	2.0 x 10 <sup>2</sup>	7.0 x 10 <sup>2</sup>
ND1	1.0 x 10 <sup>4</sup>	TNTC	TNTC	0	TNTC
ND2	TNTC	2.0 x 10 <sup>1</sup>	0	0	TNTC
ND3	1.2 x 10 <sup>4</sup>	0	4.0 x 10 <sup>2</sup>	1.0 x 10 <sup>2</sup>	6.0 x 10 <sup>2</sup>
ND4	5.7 x 10 <sup>5</sup>	8.0 x 10 <sup>2</sup>	7.0 x 10 <sup>2</sup>	0	1.0 x 10 <sup>1</sup>
ND5	4.3 x 10 <sup>5</sup>	12.0 x 10 <sup>2</sup>	9.2 x 10 <sup>2</sup>	0	3.0 x 10 <sup>2</sup>

**Legend:** TBC= total bacteria count; TCC= total coliform count; D1= Diet with 0% defatted black soldier fly larvae meal; D2 = Diet with 25% defatted black soldier fly larvae meal; D3 = Diet with 50% defatted black soldier fly larvae meal; D4 = Diet with 75% defatted black soldier fly larvae meal, D5 = Diet with 100% defatted black soldier fly larvae meal; ND1 = Diet with 0% non-defatted black soldier fly larvae meal; ND2 = Diet with 25% non-defatted black soldier fly larvae meal; ND3 = Diet with 50% non-defatted black soldier fly larvae meal; ND4 = Diet with 75% non-defatted black soldier fly larvae meal; ND5 = Diet with 100% non-defatted black soldier fly larvae meal; TNTC = Too numerous to count.

consumption except in fish fillet fed ND5 (75% non-defatted sundried BSFLM) diet that recorded too numerous to count because the value recorded is above acceptable value for human consumption at zero day of storage.

At the seventh day of storage, *Staphylococcus spp.* was detected in all fish fillets, regardless of whether they were fed defatted or non-defatted BSFLM diets, suggesting its initial presence in the fillets. This surge in microbial growth may be attributed to the nutrients provided by the dietary substrates. The isolates on the seventh day of storage comprise of diverse range of microorganisms, including *Staphylococcus spp.*, *Botrytis spp.*, *Candida spp.*, and *Bacillus spp.*, suggesting favourable environmental

conditions for their growth and potentially impacting product shelf life.

At the fourteenth day of storage, the fish fillets showed a notable decrease in microbial load (CFU/g), likely attributed to the deep-freezing of the fish fillets. This result supports the study by Masniyom *et al.* (2013), which found that the vacuum packaging technique reduced psychrotrophic bacteria in tilapia fillets compared to fish fillet samples air-packaged. According to the National Academy of Science (1985), total coliform count serves as an indicator of the hygienic conditions during the catching, handling, processing, and distribution of fish and fishery products. Neither the treatments nor the storage period had a significant impact ( $p > 0.05$ ) on total coliform counts

**Table 8.** Isolated organisms of *C. gariepinus* fillets fed defatted and non-defatted sundried black soldier fly larvae meal diets at Day twenty-first storage.

Treatments (%)	Bacterial	Mould	Yeast
D1	<i>Staph spp, Bacillus, Kleb</i>	<i>Aspergillus spp</i>	<i>Saccharomyces spp</i>
D2	<i>Bacillus</i>	-	<i>Saccharomyces spp</i>
D3	<i>Bacillus, E. coli, Kleb spp, Staph spp,</i>	-	<i>Candida spp</i>
D4	<i>Staph spp, Kleb</i>	-	<i>Saccharomyces spp, Candida spp</i>
D5	<i>Bacillus, Kleb, E. coli,</i>	<i>Botrytis spp,</i>	<i>Candida spp</i>
ND1	<i>Bacillus, Staph spp, Kleb, E. coli</i>	-	<i>Candida spp, saccharomyces spp</i>
ND2	<i>Bacillus, Staph spp</i>	-	<i>Candida spp, saccharomyces spp</i>
ND3	<i>Bacillus, E. coli, Kleb,</i>	<i>Botrytis spp</i>	<i>Candida spp, Saccharomyces spp</i>
ND4	<i>Kleb, Bacillus, Staph spp</i>	-	<i>Saccharomyces spp, Candida spp,</i>
ND5	<i>Bacillus, Staph spp, Kleb</i>	-	<i>Saccharomyces spp, Candida spp,</i>

**Legend:** D1= Diet with 0% defatted black soldier fly larvae meal; D2 = Diet with 25% defatted black soldier fly larvae meal; D3 = Diet with 50% defatted black soldier fly larvae meal; D4 = Diet with 75% defatted black soldier fly larvae meal. D5 = Diet with 100% defatted black soldier fly larvae meal; ND1 = Diet with 0% non-defatted black soldier fly larvae meal; ND2 = Diet with 25% non-defatted black soldier fly larvae meal; ND3 = Diet with 50% non-defatted black soldier fly larvae meal; ND4 = Diet with 75% non-defatted black soldier fly larvae meal; ND5 = Diet with 100% non-defatted black soldier fly larvae meal.

in catfish fillets. Only the fish fillet fed the ND5 (100% non-defatted sundried BSFLM) diet showed growth of total coliforms on the 14<sup>th</sup> day of storage, whereas the other treatments exhibited no growth of coliform, likely due to the combined effects of low temperature and residual fat in the black soldier fly larvae meal. Coliforms were not detected at zero and seventh day of storage across all treatments, possibly attributed to the frost condition of the fish fillet and pre-processing rinsing with brine solution. While at the fourteenth day of storage, total coliform counts were notably higher ( $p < 0.05$ ) in fish fillet fed the ND5 (100% non-defatted sundried BSFLM) diet compared to other treatments, but still within the permissible limit for human consumption. In addition, *Salmonella spp.* was only detected in fish fillet fed ND5 (100% non-defatted sundried BSFLM) diet on the fourteenth day of storage, which may likely due to poor handling or mismanagement during processing, as *Salmonella spp.* is typically associated with polluted environments (Gerges *et al.*, 2016).

At the twenty-first day of storage, total coliforms were detected across the treatments except in fish fillets fed D2 (25% defatted sundried BSFLM) and ND2 (25% non-defatted sundried BSFLM) diets. However, total coliform count was recorded as too high in fish fillet fed ND1 (0% non-defatted sundried BSFLM), the recorded values were above the permissible limit for human consumption reported by Gerges *et al.* (2016). This finding is consistent with Rimoldi *et al.* (2024), who identified *Staphylococcus spp.* and *Bacillus spp.* in European Seabass fed poultry by-product meal and insect exuviae. Fungi, including yeast and mould, are eukaryotic microorganisms commonly found in nature. They can be part of a food's normal microbiota or even contaminate the food, leading to changes in taste, colour, and smell. Moreover, fungi can facilitate the growth of pathogenic bacteria, leading to increased production of harmful mycotoxins, infections,

and allergic reactions in humans (Prado-Toledo *et al.*, 2022). Prado-Toledo *et al.* (2022) reported that the food preservation methods that inhibit bacterial growth can paradoxically favour fungal and yeast contamination, impacting the sensory quality of fresh, semi-processed, and processed foods. Mould counts in refrigerated fish fillets fed defatted and non-defatted BSFLM were significantly lower than yeast counts throughout the storage days. The counts in this study (ranging from 3.24 to 9.32 log<sub>10</sub> CFU/g) align with Farag *et al.* (2025) reports. Yeast and mould presence can compromise food quality, leading to deterioration, nutrient loss, and reduced product value. The fish fillets' quality assessments revealed the significance of defatted and non-defatted sundried BSFLM diets to *C. gariepinus*.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## Conclusion

Based on the microbial findings in this research, it is recommended to limit the storage duration of catfish fillets fed diets containing sundried BSFLM, both defatted and non-defatted, to prevent spoilage and food safety issues.

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