Research Article

Baker's Yeast-Supplemented Black Soldier Fly Larvae as a Sustainable Fishmeal Alternative in Nile Tilapia Diets: Impacts on Growth, Health and Gut Microbiota

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Abstract | The inclusion of fishmeal in aquaculture poses sustainability issues due to the reliance on wild fish. Therefore, scientists and aquafarmers continue to search and find alternative sources of protein. This study aimed to investigate non-defatted black soldier fly larvae meal supplemented with baker's yeast, *Saccharomyces cerevisiae*, as a total replacement for fishmeal in the diets of Nile Tilapia, *Oreochromis niloticus*. This study highlights the potential of black soldier fly larvae meal supplemented with baker's yeast as a sustainable and cost-effective alternative to fishmeal, promoting environmental conservation and enhancing aquaculture productivity. Three experimental diets were formulated: T1 based on fishmeal, T2 based on BSFL with baker's yeast supplementation and T3 was BSFL without yeast. Growth performance, feed utilization, body composition, haematology, gut histomorphology and gut microbiota of juvenile Nile Tilapia fed these diets for 60 days were evaluated. The results showed that T2 had comparable growth and nutrient utilisation to T1, while T3 showed inferior performance. Yeast supplementation improved feed conversion efficiency, intestinal morphology, and protein and lipid utilization. The replacement did not affect negatively the fish blood parameters. Importantly, yeast also improved gut microbial balance, implying a probiotic benefit. This work demonstrates the promise of yeast-enriched BSFL meal compared to BSFL meal alone as a sustainable alternative to fishmeal for aquaculture that promotes the growth and health of Nile Tilapia. These results contribute to sustainable aquaculture by reducing reliance on wild fish. However, scaling up production and consumer acceptance remain challenges for widespread adoption.

Keywords | Nile tilapia, Probiotic, *Saccharomyces cerevisiae,* BSFL *Hermetia illucens,* Fish meal replacement

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INTRODUCTION

Aquaculture, the farming of fish, shellfish and other aquatic organisms, has undergone significant changes in recent decades and is currently a very important activity in the context of global food security and environmental sustainability (Boyd *et al*., 2022; FAO, 2022). Once considered a minor industry, aquaculture has expanded rapidly, driven by technological innovations in the areas of breeding, nutrition and disease control (Naylor *et al*., 2021; Verdegem *et al*., 2023). Therefore, modern aquaculture techniques focus on the practice of sustainable farming with reduced environmental impact through aquaculture practices and greater efficiency in the use of resources (Naylor *et al*., 2021). Aquaculture provides a sustainable way to meet the high and growing demand for seafood, as wild fish populations are overexploited and negatively affected by climate change (Cheung *et al*., 2023). It reduces pressure on natural ecosystems and provides high-quality protein reliably (Verdegem *et al*., 2023). With the current development, the aquaculture industry is able to offer significantly balanced and environmentally friendly food systems to the future world (Verdegem *et al*., 2023). Nile tilapia is considered one of the most important freshwater fish and is widely farmed worldwide due to its rapid growth rate, adaptability to various environmental conditions and palatability (Ntakirutimana *et al*., 2023; Tabassum *et al*., 2021). The sustainability of aquaculture production is mainly limited by the availability of feed ingredients that are both environmentally friendly and nutritionally balanced (Munguti *et al*., 2023). The aquaculture sector has faced increasing challenges in recent times to meet the ever-increasing demand for fish protein without negatively impacting environmental concerns and to ensure the sustainability of feed resources (Boyd *et al*., 2022). Among the most significant challenges for aquaculture is the reliance on fishmeal, which is a high-quality protein source mainly from wild-caught marine fish, which poses a major food security problem, raises sustainability issues and raises economic problems due to overfishing and fluctuating availability (Kariuki *et al*., 2024; Moutinho *et al*., 2022). In fact, fishmeal production carries significant ecological and economic concerns due to overfishing of forage fish species (Japanese anchovy, Chilean jack mackerel, anchoveta, chub mackerel, etc.) and resulting marine biodiversity decline. Moreover, the price of fishmeal is susceptible to market demand, climate impacts, and availability of resources, causing financial volatility within aquaculture operations (Deutsch *et al*., 2011; Diana, 2009; MR and M Halwart, 2009). As per the report of FAO (2022), the price of farmed fish is expected to climb by 2030, with the price of fishmeal expected to rise by around 11%. It has therefore become urgent to seek alternative sources of protein feed that are nutritionally adequate for fish feeding and environmentally friendly (Moutinho *et al*., 2022). Among the promising alternatives to traditional fishmeal, black soldier fly (*Hermetia illucens)* larvae have attracted considerable attention in recent years for their high nutritional value, rapid growth and ability to convert organic waste into high-quality protein biomass (Moutinho *et al*., 2024; Munguti *et al*., 2023). Black soldier fly larvae contain an exceptionally high proportion of essential amino acids, lipids and minerals. The replacement of fish meal by BSFL meal in aquaculture is also economically more profitable than relying on traditional fish meal (Mathai *et al*., 2024). These factors make BSFL particularly useful as protein sources for aquaculture and other types of livestock farming (Kari *et al*., 2023). As shown by studies by Fontes *et al*. (2019) and Monteiro *et al*. (2023), the digestibility of diets based on non-defatted BSFL is relatively low compared to traditional fishmeal diets in Nile tilapia and Tambaqui respectively. This has led the majority of researchers to believe that BSFL should only partially replace fishmeal. As for Tippayadara *et al*., (2021) replacement up to 100% did not have any negative impact on the health of Nile Tilapia, but the maximum performance was observed at 20 and 40% BSFL dose. However, Kari *et al*., (2023) conducted a feeding trial on *Betta splendens* and found that 13% BSFL is the optimum dose to replace fish meal and that performance starts to deteriorate at higher doses. Zhao *et al*. (2023) also concluded that replacing 14% of FM by defatted BSFL does not cause any significant difference in Turbot performance, but that the same dose negatively affects performance if the BSFL is not defatted. Similar findings were made by Fontes *et al*. (2019) and Monteiro *et al*. (2023). All of these authors agree that issues with the digestibility of specific compounds in the insect are the cause of the decline in fish performance at high BSFL dosages. With its probiotic properties, baker's yeast (*S. cerevisiae*) has been shown to stimulate enzyme production in the intestine and thus improve digestibility in hosts (Boonanuntanasarn *et al*., 2019; Siddik *et al*., 2021). In fact, the cell wall of baker's yeast comprises β-glucans and mannans that are believed to induce the immune system and improve nutrient digestibility (Darafsh *et al*., 2020). Additionally, it may foster the proliferation of other beneficial bacteria in fish's digestive tract which enhances the breakdown of the majority of nutritional components.

Therefore, supplementation of baker's yeast as probiotic in fish diets leads to improved feed conversion efficiency and nutrient utilization. Baker's yeast is also a probiotic known to contain high levels of protein, a rich amino acid profile that improves feed digestibility and immune functions of aquatic organisms (Baker *et al*., 2022). In fish feed formulations, the nutritional profile of black soldier fly larvae (BSFL) can be significantly improved by supplementing the mix with probiotic *S. cerevisiae*, creating a complete and sustainable substitute for traditional fishmeal. This combination could provide a balanced nutritional profile, improving nutrient digestibility and absorption. To our knowledge, no study has attempted the approach of supplementing BSFL with baker's yeast in Nile tilapia. This study is therefore designed to contribute to the existing knowledge gap on the use of BSFL as an alternative to fishmeal in juvenile Nile tilapia. The growth performance parameters, feed utilization efficiency, biochemical body composition, microbial load and hematological parameters of Nile tilapia fed diets containing fish meal as main source of protein, BSFL meal as source of protein, and BSFL with baker's yeast supplementation diet are evaluated in a feeding trial.

MATERIALS AND METHODS

Experimental Diets and Study Design

Three experimental diets were formulated to assess the importance of supplementing black soldier fly larval meal with known doses of baker's yeast (*S. cerevisiae*) as a complete alternative to fishmeal in the diets of juvenile Nile tilapia. The control diet (T1) included conventional fishmeal as the main protein source, the experimental diet T2 had full-fat BSFL meal as the main protein source but supplemented with a known dose of 4 g kg^{-1} of baker's yeast (10^{10} CFU g^{-1}) (Islam *et al*., 2021; Opiyo *et al*., 2019), while T3 was a diet where fishmeal was replaced by full-fat BSFL meal without probiotic supplementation. Following laboratory analysis of the crude protein contents of the two meals, Pearson square was used to calculate the amount of BSFL meal with a crude protein content equivalent to that of fish meal. The other components of the diets were similar in the three treatments and are summarized in Table 1 with their proportion and the proximal composition of the pellets formed. The feeding trial was conducted in a randomized complete block design with each diet represented in triplicate. Juvenile Nile Tilapia (10.19±0.70g; 4.88±0.98 cm) were collected from the Central Institute of Fisheries Technology (ICAR-CIFT) Cochin, Kerala and stocked for a 2-week acclimation period. After this period, they were randomly allocated to 100 L experimental tanks in the enclosures of the School of Industrial Fisheries, Cusat. Tanks containing dechlorinated tap water were stocked at a density of 12 fish per tank with continuous water aeration. The random allocation ensured uniformity of initial body weight and size across treatment groups. Fish were fed their respective experimental diets twice daily at a feeding rate of 5% of body weight daily. The experimental feeding trial was extended for a period of 60 days. During this period, culture water quality parameters were maintained at optimal values (Table 2) by removing waste and feed residues and renewing part of the water with waste.

Growth Performance, Survival and Feed Utilization

All fish in each tank were weighed and measured fortnightly to record weight and length data to assess growth progress. Weight was measured using an electric balance, total length was taken using a scale graduated in mm under safe handling conditions. At the end of the trial period, following parameters were assessed: Survival rate as percentage of survived fish from each tank, final total length (FTL), final body weight (FBW), net weight gain (NWG), weight gain (WG), average daily weight gain (ADWG), specific growth rate (SGR), feed conversion ratio (FCR) and feed conversion efficiency (FCE). Calculations were done as follow:

$$Survival\ rate = \frac{Final\ number}{Initial\ number} \times 100$$

NWG (g) = Final weight – Initial weight

$$WG\ (\%) = \frac{(Mean\ final\ weight - mean\ initial\ weight)}{Mean\ initial\ weight} \times 100$$

$$ADWG\ (g/fish/day) = \frac{(Mean\ final\ weight - mean\ initial\ weight)}{trial\ duration\ (days)}$$

$$SGR\ (\%/day) = \frac{(\ln(final\ weight) - \ln(initial\ weight))}{trial\ duration\ (days)} \times 100$$

$$FCR = \frac{total\ dry\ feed\ intake\ (g)}{total\ fish\ weight\ gain\ (g)}$$

$$FCE = \frac{total\ fish\ weight\ gain\ (g)}{total\ dry\ feed\ consumed\ (g)}$$

Gut and Water Microbial Load

The total microbial count (TBC) analysis of culture water and fish guts was performed at the beginning and end of the experimental period to monitor changes in microbial populations using plate count agar (standard methods agar) following Tabassum *et al*. (2021). At the end of the feeding trial, two fish were collected per tank and two water samples per tank at both sampling times. Water samples were collected in sterile containers, refrigerated at 4 °C until analysis. Guts were aseptically dissected from euthanized fish and vortexed in sterile saline to homogenize the contents before storage in a 4 °C refrigerator. Samples were serially diluted and two hundred micrometres (0.2 ml) of each dilution were inoculated in triplicate for microbial enumeration. Cultures were incubated at 30 °C for 24–48 h and then counted for CFU. Each sample was subjected to the plate spread technique performed in triplicate for accuracy. The control included sterile saline blanks as negative controls.

Fish Biochemical Body Composition

Body biochemical composition analysis was carried out on the muscle tissues of dissected fish to determine crude protein, crude lipid, ash, and moisture content. Muscle samples from fish used in previous section were taken and were analysed using standard methods described by the AOAC (2023). Crude protein content was derived using the Kjeldahl method, as measuring the amount of nitrogen gives an estimate of the protein content. Ash content was determined by ashing the muscle samples in a muffle furnace at 550 °C until all the organic matter was burnt, leaving only the inorganic ash. The crude lipids were extracted by the Soxhlet extraction method. Moisture content was assessed by drying the samples in an oven for a specified time at 105 °C until constant weight was obtained. Measurements were performed in triplicate to ensure precision.

Table 1: Proportions of diets ingredients and proximate composition of formulated pellets.

Ingredients (g)		Fish meal	Baker's Yeast	BSFLM	Soybean meal	Rice bran	Wheat flour	Cassava flour	Multivitamin and minerals*	Sunflower oil
	T1	512	0	0	355	56	77	20	15	40
	T2	0	4	925	355	56	77	20	15	40
	T3	0	0	925	355	56	77	20	15	40
Pellets Proximate analysis (%) **		Crude Protein	Crude Lipid	Moisture	Ash					
	T1	41.58±2.32	13.24±0.71	8.71±0.62	12.87±0.34					
	T2	41.32±1.41	15.63±0.54	8.41±1.35	11.44±1.18					
	T3	40.99±2.18	15.64±0.73	8.17±0.37	12.22±0.81					

*Each capsule used contained Vitamin A (as Vitamin A Concentrate Oil IP) 5000 IU, Vitamin D3 (Cholecalciferol IP) 400 IU, Vitamin E (Tocopheryl Acetate IP) 15mg, Vitamin B1, IP 5 mg, Vitamin B2 IP 5 mg, Nicotinamide IP 45 mg, D-Panthenol IP 5 mg, Vitamin B6 IP 2 mg, Vitamin C IP 75 mg, Folic Acid IP 1000 mcg, Vitamin B12 IP 5 mcg, Dibasic Calcium Phosphate IP 70 mg, Copper Sulfate Pentahydrate BP 0.1 mg, Manganese Sulfate Monohydrate BP 0.01 mg, Zinc Sulphate Monohydrate IP 28.7 mg (equivalent to 10.4 mg of elemental Zinc), Potassium Iodide IP 0.025 mg, Light Magnesium Oxide IP 0.15 mg. ** Values are presented as mean ± SD.

Intestine Histomorphology

Histomorphological analysis of the intestine was performed using three fish per treatment at the end of the experimental period. Fish were dissected and the proximal intestine was carefully excised and immediately fixed in 10% neutral buffered formalin to preserve tissue integrity. After fixation, intestinal samples were dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin. Thin sections (4–6 µm) were cut using a microtome and mounted on glass slides. Sections were then stained with haematoxylin and eosin (HandE) to highlight tissue structure. After staining, the slides were examined, images were captured, and histological features such as villi height and width, and muscle layer thickness were measured using an upright microscope (LEICA DM6 B) coupled with a digital camera (LEICA DFC450 C) and an image analysis software LAS X (LASX Office 1.4.7 28921). For each fish intestine, two sections were considered for measurement. And at each section, three villi were chosen for height and width measurement and three areas around the section were chosen for muscular thickness measurement. The measurements obtained were subjected to statistical analysis for comparison.

Fish Haematology

Blood samples were collected from two fish per tank at the end of the experimental period. Blood was collected using syringes pre-coated with EDTA to prevent clotting. Complete blood cell (CBC) was analyzed using the electrical impedance method, VCS (volume, conductivity, and scatter) and photometry (Bruegel *et al.*, 2015) by passing the blood samples through an automated hematology analyzer (Beckman COULTER® GEN. S™), which accurately quantifies cell counts, cell sizes, and hemoglobin content by passing the blood through the analyzer where electrical signals and light scattering patterns are measured. Red blood cell and white blood cell counts, hemoglobin, hematocrit, mean platelet volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, mean corpuscular volume, platelet or thrombocyte count, and differential leukocytes namely neutrophils, lymphocytes, monocytes, eosinophils and basophils were measured.

Statistical Analysis

Data were subjected to one way ANOVA using SPSS software (IBM SPSS Statistics 25) to determine significant differences between treatment groups. Mean values and standard deviations were calculated for each parameter, and Tukey's post-hoc test was employed to compare means between treatments at $p < 0.05$.

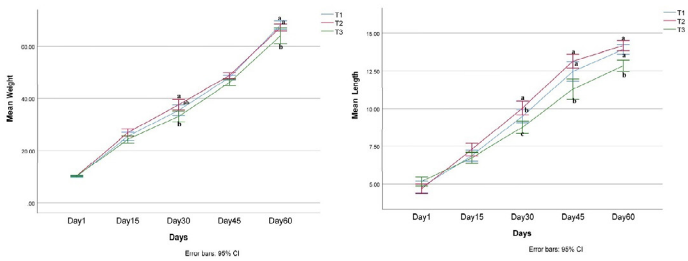

Figure 1: Growth evolution of Nile Tilapia fed with fish meal (T1), BSFL meal and yeast (T2) and BSFL meal (T3) diets (a, b, and c are indicatives of statistical differences).

RESULTS AND DISCUSSION

Growth Performance and Feed Utilisation

The results of the analysis on growth performance parameters, feed utilization and survival rate are summarized in Table 3 while the graphs in Figure 1 show the evolution of fish growth during the experimental period. A significant difference ($p<0.05$) was observed for all parameters related to growth performance and feed utilization. Survival rate was higher in T1 and lower in T3. Post-Hoc test between groups showed that growth and feed utilization parameters are significantly different ($p<0.05$), especially between T1 and T3. FTL values are higher in T2 followed by T1 and those of T3 are lower. FBW, NWG, WG, ADWG, FCE and SGR values are higher in T1 group followed by T2 and those of T3 are significantly lower. T3 shows highest FCR values followed by T2 and T1. The results of the present study showed that growth performance and feed utilization were significantly affected by diet type, mainly in the comparison between the fishmeal diet T1 and the BSFL diets T2 and T3. On the other hand, the full replacement of fish meal by BSFL meal did not significantly affect the growth performance of Nile tilapia in the studies by Mathai *et al.* (2024) and gilthead seabream juveniles (Moutinho *et al.*, 2022). Intraspecific and interspecific disparities are observed in studies results on the effect of BSFL on fish performance. In their study Mikołajczak *et al.* (2022) showed that the replacement of 25 or 50 % of fish meal by BSFL did not have a negative impact on growth of brown trout. However, the results of the study conducted by Zhao *et al.* (2023) showed that replacement of fish meal by full fat BSFL meal affected negatively the growth performance of juvenile Turbot. Similarly, in the feeding trial by Kariuki *et al.* (2024) the highest level (75%) replacement of fish meal by BSFL meal had lowest growth performance in Nile Tilapia but without a significant difference. Black soldier fly larvae (BSFL) efficacy may vary across fish species due to species-specific factors such as digestive physiology, gut microbiota composition, and dietary requirements. Carnivorous fish may experience lower BSFL protein digestibility due to their specialized digestive enzymes (Colombo, 2020). On the other hand, organisms with diverse enzymatic profiles and microbial communities like tilapia or catfish can efficiently utilize BSFL-derived nutrients (Nogales-Mérida *et al.*, 2019). The inclusion level and form of BSFL in diets also affect digestibility (Colombo, 2020; Nogales-Mérida *et al.*, 2019). This perspective deserves an extensive specific study. Environmental factors like water temperature and feeding behavior also influence BSFL diet effectiveness (Colombo, 2020). Considering Tilapia separately, it is evident that the low growth performance of fish fed with non-defatted BSFL diets has an origin in the fact that the digestibility of these diets is relatively low compared to traditional fishmeal diets as shown in the studies by Fontes *et al.* (2019) and Opiyo *et al.* (2019). In fact, insects contain chitin that may harden the digestion for fish (Mikołajczak *et al.*, 2022; Zhao *et al.*, 2023). Therefore, in the present study, the yeast-supplemented BSFL had a growth performance similar to that of the fishmeal group, while the unsupplemented BSFL diet, T3, had a lower performance. This would imply that baker's yeast is involved in the increase in feed conversion ratio and specific growth rate noted above in similar studies (Islam *et al.*, 2021; Sadeghi *et al.*, 2022). The presence of yeast would improve nutrient availability, absorption, and digestion facilitated by the known probiotic properties of yeast to aid in the production of enzymes, maintaining good gut health (Jahan *et al.*, 2021; Siddik *et al.*, 2021). In fact, baker's yeast has been used widely for probiotic purposes, effectively improving nutrient uptake and gut health in fish (Baker *et al.*, 2022). Extracellular enzymes from the yeast include proteases, lipases, and amylases that break down complex nutrients, making their bioavailability better (Baisakhi *et al.*, 2024; Jahan *et al.*, 2021). Moreover, yeast helps in maintaining gut homeostasis through competition against the pathogenic bacteria and helping in the establishment of beneficial microbial communities (Opiyo *et al.*, 2019; Baisakhi *et al.*, 2024). Yeast cell walls contain β-glucans and mannans, which stimulate the immune system to increase resilience to stress and disease (Boonanuntanasarn *et al.*, 2019). Additionally, yeast provides essential B vitamins and minerals that would otherwise be limiting, thereby improving growth and metabolic efficiency (Demirgul *et al.*, 2022). As justified by the results on microbiota and intestinal morphology in the present study, these combined benefits place baker's yeast as a valuable additive in aquafeeds, which results in better growth performance, improved feed conversion ratios, and healthier fish populations.

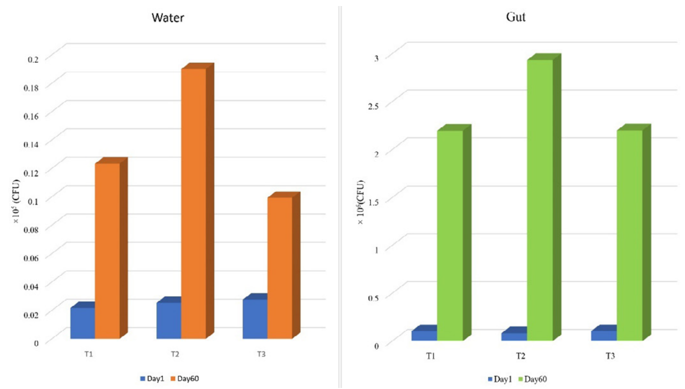

Figure 2: Microbial load in rearing water and gut of Nile Tilapia fed with fish meal (T1), BSFL meal and yeast (T2) and BSFL meal (T3) diets before and after the feeding trial.

Effect on Gut and Water Microbial Load

The impact of experimental diets T1, T2 and T3 on the bacterial community in the gut and in the effluent water of the tanks is presented in Figure 2 which shows the bacterial load at the beginning and end of the experimental period. The total number of bacteria in the gut of the fish and the effluent (rearing water) highly increased in the group T2 than the groups T1 and T3 but there was no significant difference ($p > 0.05$). Similar results were found in the study where the inclusion of BSFL meal as alternative to fish meal did not significantly affect the intestinal microbiota of brown trout (Mikołajczak *et al.*, 2022). However, the research on golden pompano showed that BSF pulp supplementation reduce the number of operational taxonomic units in the fish intestine (Li *et al*., 2023). Similarly, Zhao *et al*. (2023) concluded that the inclusion of non-defatted BSFL meal altered the abundance of intestinal microbiota in juvenile turbot. Indeed, insects, especially the black soldier fly, besides providing nutritional and bioactive compounds (Messina *et al*., 2019), are known to have the ability to transfer potential antimicrobial factors, what may be the reason of reduced microbiota when they are added to a diet (Lee *et al*., 2022; Xia *et al*., 2021; Yildirim-Aksoy *et al*., 2022). On the other hand, research have shown that *S. cerevisiae* can affect the gut microbiota, by for example increasing beneficial bacteria and reducing or suppressing pathogenic ones (Baker *et al*., 2022). That property leads to improved digestive and immune functions, reducing disease incidence (Boonanuntanasarn *et al*., 2019). For instance, it has been shown that dietary supplementation of baker's yeast in rohu (Jahan *et al*., 2021) and juvenile barramundi (Siddik *et al*., 2021) increases the number of beneficial bacteria, supports nutrient absorption and digestion, and reduces harmful bacteria. It further enriches microbial diversity, hence allowing for the efficient running of digestion and immunity (Baker *et al*., 2022). All the effects of yeast on gut microbiota are therefore mainly attributed to its bioactive components, which function as prebiotics by reshaping the microbial community in the gut (Siddik *et al*., 2021). Hence, the lack of statistically significant difference in this study may be the result of the microbial balance due the actions of both BSFL and baker's yeast and suggests further specific research that incorporates microbiota profiling to identify specific bacterial species or groups for long-term changes in microbial populations.

Impact on Fish Body Composition

The results of the analyses on the biochemical composition of the muscles of fish fed with T1, T2 and T3 diets are summarized in Table 4. Moisture and Ash composition showed no significant difference between groups ($p > 0.05$). The significant difference between groups was observed in crude lipid and crude protein composition ($p < 0.05$). The composition in crude lipid was higher in T3 followed by T2 and T1. Interestingly, crude protein content was highest in the T2 group and lowest in the T3 group, with T1 falling in between. This therefore means that although BSFL alone could not be sufficient to meet the protein requirements of Nile tilapia compared to fishmeal. It is stated that the composition of BSF is about 50~60% crude protein, making it a good alternative to fishmeal (Mohan *et al*., 2022) and the replacement of up to 50% of fishmeal with BSFL meal has no significant effect on crude protein and lipid content (Abdel-Tawwab *et al*., 2020). However, the full replacement of fish meal by BSFL meal in this study showed lower crude protein content than fish meal diet and then the diet which includes baker's yeast. Our results corroborate those of Siddaiah *et al*. (2023) that showed that as the replacement level of BSFL meal increases, the crude protein level decreases in Snakehead juveniles. Hence, yeast supplementation increases protein utilization. This can also be evidenced by the studies of Owatari *et al*. (2022) and Darafsh *et al*. (2020) where inclusion of *S cerevicieae* in the diet increased crude protein content in *O. niloticus* and *Acipenser persicus* respectively. Yeast, particularly *S. cerevisiae*, is known to enhance nitrogen assimilation by providing bioactive compounds such as nucleotides, amino acids, and peptides, which support protein synthesis and improve metabolic efficiency (Jach *et al*., 2022). Furthermore, yeast supplementation can enhance the activity of proteolytic enzymes, facilitating the degradation and absorption of dietary proteins (Baisakhi *et al*., 2024). This is consistent with findings that *S. cerevisiae* can modulate the gut microbiota, creating a favourable environment for the production of short-chain fatty acids and other metabolites that promote nitrogen retention and utilization (Abid *et al*., 2022). Furthermore, the higher lipid content in T3 may indicate that fat digestion and assimilation when yeast is not added is inefficient; therefore, yeast improves lipid metabolism. The direct effect of *S. cerevisiae* in lipid digestion is not well documented, but it is known that yeast or its compounds like β-glucan has the ability to produce emulsifying agents that adhere to break down aggregates of fats, oils, and sludge (Tao *et al*., 2023).

Table 2: Water quality parameters in Nile tilapia fed with fish meal (T1), BSFL meal and yeast (T2) and BSFL meal (T3) diets (DO: dissolved oxygen; BDL: below detectable level). All parameters are not statistically different among treatments.

Parameters	T1	T2	T3	p-value
Temperature (°C)	26.92± 0.59	26.80± 0.58	26.90± 0.60	0.242
pH	7.49± 0.87	7.40± 0.85	7.52± 0.80	0.515
Salinity (ppt)	0±00.00	0±0.00	0±0.00	-
Alkalinity (mg/L)	38.75± 15.05	42.50± 18.70	32.50± 14.14	0.468
Carbonate (mg/L)	0±00.00	0±0.00	0±00.00	-
Bicarbonate (mg/L)	38.75± 15.05	42.50± 18.70	32.50± 14.14	0.468
Hydroxide (mg/L)	0±00.00	0±0.00	0±0.00	-
Total Hardness (mg/L)	43.75± 15.52	47.50± 13.88	40.00± 16.03	0.620
Calcium (mg/L)	10±00	10±00	10±00	-
Magnesium (mg/L)	30.00± 16.03	30.00± 16.03	37.50± 13.88	0.539
Ammonia (mg/L)	0.066± 0.052	0.027± 0.021	0.092± 0.084	0.107
Nitrite (mg/L)	0.225± 0.06	0.043± 0.041	0.127± 0.02	0.563
Sulfide (mg/L)	BDL	BDL	BDL	-
DO (mg/L)	8.2± 0.8	7.9±0.4	8.3±0.5	0.444

Values are presented as mean ±SD.

Table 3: Growth, feed utilization and survival parameters found in different treatments.

Parameters	T1	T2	T3	p-value
ITL cm	4.79±1.17	4.68±0.93	5.16±0.89	0.11
FTL cm	13.92±0.89[a]	14.16±0.95[a]	12.84±1.04[b]	0.00
IBW g	10.02±0.87	10.28± 0.65	10.19± 0.64	0.25
FBW g	68.23±4.51[a]	67.14± 3.67[ab]	63.93 ± 8.38[b]	0.008
NWG g	58.35±0.86[a]	56.86± 0.22[ab]	53.80± 2.64[b]	0.034
WG %	581.92±4.33[a]	553.13±13.43[ab]	524.11±29.49[b]	0.027
ADWG	0.97± 0.01[a]	0.94± 0.00[ab]	0.89± 0.04[b]	0.034
SGR	3.20± 0.01[a]	3.12± 0.03[ab]	3.05± 0.07[b]	0.031
FCR	1.52± 0.02[a]	1.56± 0.01[ab]	1.65± 0.08[b]	0.045
FCE	0.65± 0.01[a]	0.64± 0.00[ab]	0.60± 0.02[b]	0.03
Survival rate (%)	100± 00	97.22 ± 4.81	96.29 ± 6.05	0.25

Values are presented as mean ±SD. Values with the same superscript letter in the same row are not significantly different (there was no significant difference between the groups for values without superscript letter).

Effect on Intestinal Histomorphology

As expressed by the results summarized in Table 5, fish under the T2 diet expressed significantly ($p<0.05$) higher values of VH and MT. However, there was no significant difference between the groups in VW ($p>0.05$). Intestinal villus density was also found to be very high in fish under the T2 diet followed by those under the T1 diet while fish under the T3 diet had very low villus density (Figure 3). Indeed, high-dose replacement of fishmeal with BSFL meal would negatively affect the structure of the fish intestine (Huang *et al*., 2022). This is also supported in the results of the study by Kari *et al*. (2023) in which replacing up to 19% of fishmeal with BSFL meal had a positive impact on villi length and width, but from 24% replacement these gut structures decline drastically. However, it has been shown that the addition of baker's yeast (*S. cerevisiae*) to the diet influences the growth of histo-morphological structures in the fish intestine. For instance, Islam *et al*. (2021) showed that the addition of 4g kg^{-1} of baker's yeast in the diet increased the surface area, length, and width of the villus in the intestine of Nile tilapia. Similarly, the width and length of epithelial folds in the intestine of Nile tilapia were positively affected by the addition of *S. Cerevisiae* in the diet (de Moraes *et al*., 2022). Jahan *et al*. (2021) also found that baker's yeast significantly increased the length and width of the villus as well as the depth of the crypts of rohu intestine. These structures positively impacted by this probiotic are directly or indirectly linked to better nutrient absorption and better intestinal health in host species (Darafsh *et al*., 2020). In addition, increased crypt depth and intestinal wall thickness indicate improved cell turnover and a healthier intestinal environment, which helps maintain the integrity and functionality of the intestine (Islam *et al*., 2021). The epithelial cells covering the villi are rich in transporters and enzymes essential for breaking down and absorbing nutrients (Wang *et al*., 2021). Increased VH and density enhance the availability of these active sites, speeding up the diffusion and active transport of nutrients into the bloodstream or lymphatic system. Taller and denser villi ensure better exposure of the feed to digestive enzymes and gut microbiota. This can enhance nutrient breakdown and release, further improving absorption efficiency (Ringø *et al*., 2022). Increased VH and density enhance the number and activity of goblet cells, promoting a robust mucus layer that acts as a physical barrier, delivers antimicrobial agents, and facilitates immune signalling to protect the intestine from pathogens (Merrifield *et al*., 2010). The large thickness of the intestinal muscular layer facilitates the intestine to make contractions during digestion (Jiao *et al*., 2023). These histological improvements likely contributed to better growth and survival outcomes observed in the present study by optimizing nutrient absorption and enhancing intestinal integrity, thereby supporting overall health and resilience. This ensures good intestinal health of the host by adding baker's yeast to the BSFL meal diet as a total replacement for fish meal.

Table 4: Results of the proximate analysis on the biochemical composition of fillets of Nile Tilapia fed by T1, T2 and T3.

Treatment	T1	T2	T3	p-value
Moisture %	72.60±1.61	73.35±1.70	74.23±2.08	0.57
Ash %	15.72±0.49	15.67±0.34	16.31±0.31	0.18
Crude lipid %	8.84±0.62[a]	11.32±1.15[b]	14.75±0.74[c]	0.001
Crude Protein	67.43±2.08[ab]	67.80±1.05[a]	64.27±0.89[b]	0.045

Values are presented as mean ±SD. Values with the same superscript letter in the same row are not significantly different (there was no significant difference between the groups for values without superscript letter).

Impact on Fish Haematology

Analysis of hematological parameters is important in the assessment of fish health, particularly under different feeding regimes as it reflects immune and physiological responses (Jahan *et al*., 2021). At the end of the trial, the complete blood cell (CBC) test of fish fed diets T1, T2, and T3 was assessed. The findings on its parameters are shown in Table 6. The distribution of the main components of differential leucocytes count (DLC) among the three treatments is depicted in Figure 4. The significant difference between the different treatments was observed only for platelet and neutrophil count ($p<0.05$). All other hematological parameters showed no significant difference between the groups ($p>0.05$). The absence of significant differences between groups for hemoglobin (Hb), hematocrit (PCV), red blood cell (RBC) count and red blood cell indices (MCV, MCH, MCHC) indicates that all the diets were sufficiently adequate for oxygen transportation in Nile tilapia. Similar results were found in the studies where *S. cerevisiae* supplementation had no significant impact on red blood cells, cell volume, hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration in fish. For instance, *S. cerevisiae* diet in striped catfish (*Pangasianodon hypophthalmus*) had no statistically significant effect on the values of several hematological indices, including red blood cell count, haemoglobin and haematocrit (Boonanuntanasarn *et al*., 2019).

Table 5: Effects of T1, T2, T3 diets on the intestinal histomorphology of Nile tilapia.

Parameter	T1	T2	T3	p-value
Villi height (µm)	304.70±39.58[a]	348.88±81.34[a]	201.19±15.79[b]	0.000
Villi width (µm)	105.13±22.64	111.12±22.56	103.16±24.17	0.642
Muscular thickness (µm)	48.46±10.85 [a]	69.99±8.18 [b]	67.57±13.46 [b]	0.000

Values are presented as mean ±SD. Values with the same superscript letter in the same row are not significantly different (there was no significant difference between the groups for values without superscript letter).

Table 6: Hematological parameters of Nile tilapia fed with different diets

Parameters	T1	T2	T3	p-value
Hemoglobin (g/dL)	7.93±0.63	7.13±1.51	8.23±1.20	0.533
PCV (Hct) %	27.00±2.00	25.33±2.63	28.40±6.58	0.693
RBC mill/mm3	2.33±0.62	1.86±0.85	1.81±1.81	0.553
MCV (fL)	162.23 ±7.73	178.56 ±12.15	152.83 ±30.48	0.330
MCH (pg)	47.66±3.55	47.56±1.30	48.23±5.97	0.977
MCHC (g/dL)	29.33±3.76	27.40±2.72	30.70±3.83	0.541
RCD %	10.00±0.43	9.50±0.17	11.06±2.93	0.554
WBC thou/mm3	2.16±0.11	2.26±0.35	2.43±0.25	0.486
Neutrophils %	1.53±0.15[a]	11.03±6.82[b]	1.72±1.16[a]	0.043
Lymphocytes	96.86±0.70	72.73±11.50	82.32±16.06	0.103
Monocytes %	1.32±0.62	10.43±3.01	15.75±14.82	0.204
Eosinophils %	0.25±0.20	5.80±5.14	0.19±0.18	0.098
Basophils %	0±00	0±00	0±00	-
Platelet Count (thou/mm3)	11.0±1.73[a]	5.66±1.52[b]	7.33±1.15[ab]	0.012
MPV (fL)	8.56±0.65	9.73±1.74	8.06±0.32	0.238

Values are in presented as mean ±SD. Values with the same superscript letter in the same row are not significantly different (there was no significant difference between the groups for values without superscript letter).

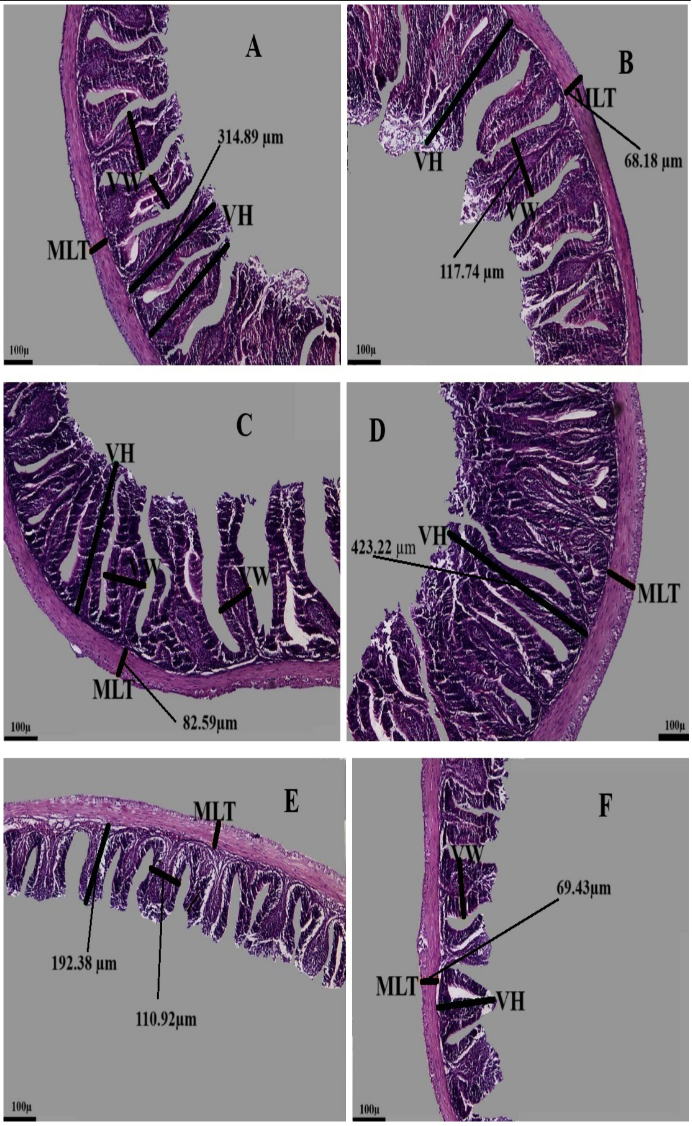

Figure 3: Histomorphological structure of the proximal intestine of Nile tilapia fed by T1 (A and B), T2 (C and D) and T3 (E and F). VH, VW, and MLT indicate the measurement of villi height, villus width, and muscular layer thickness, respectively.

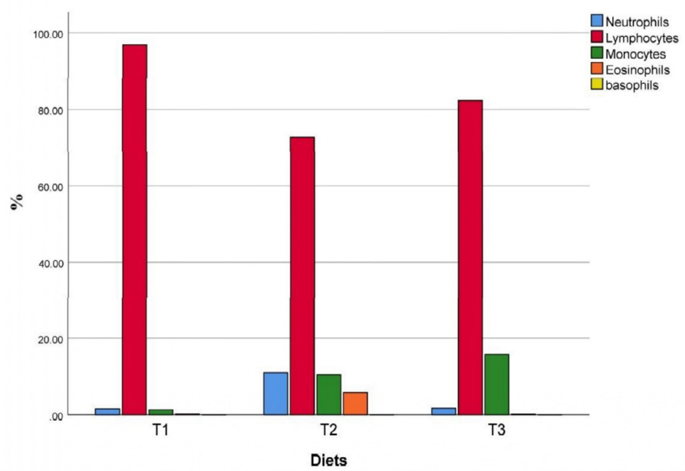

Figure 4: Differential leukocyte count (DLC) of Nile tilapia fish fed with fish meal(T1), BSFL meal and yeast (T2) and BSFL meal (T3) diets.

Similarly, it was shown that the replacement of fish meal by BSFL meal does not significantly affect the red blood cell, hemoglobin, hematocrit, MCV, MCH, and MCHC values of European sea bass, (*Dicentrarchus labrax*) (Abdel-Tawwab *et al*., 2020). There was no significant difference between treatments for WBC overall, and lymphocytes appear to be a dominant component of DLC throughout. This shows that replacing fishmeal with BSFL meal has no negative effect on immune response or systemic inflammation, which corroborates the results of Abdel-Tawwab *et al*. (2020). However, T2, which contained BSFL and baker's yeast, had significantly increased neutrophils indicating a possible inflammatory or immune response triggered by the BSFL diet supplemented with baker's yeast which may have immunostimulatory effects (Sadeghi *et al*., 2022). Neutrophils, phagocytic cells, are critical components of the innate immune system, and their elevated levels may indicate enhanced readiness to combat infections or respond to inflammatory triggers (Neuls *et al*., 2021). This aligns with findings by Samidjan *et al*. (2023), who demonstrated that yeast-derived components such as β-glucans can activate immune cells, including neutrophils, by binding to specific pattern recognition receptors (e.g., dectin-1). Consequently, the inclusion of baker's yeast in the T2 diet may have bolstered the fish's immune resilience, enhancing their capacity to manage pathogens or environmental stressors.

CONCLUSIONS AND RECOMMENDATIONS

Black soldier fly larvae meal enriched with baker's yeast, Saccharomyces cerevisiae, could be a promising alternative to fishmeal that could completely replace it in Nile Tilapia aquaculture diets. Yeast supplementation in BSFL diets showed improved growth performance, feed utilization, and nutrient digestibility closer to the levels observed with fishmeal diets. Yeast supplementation also showed improved gut health and gut microbiota balance, thus promoting better fish health. In contrast, the results obtained by the diet without BSFL supplementation were not at optimal levels, making yeast crucial in optimizing BSFL diets. The results of this study support the use of BSFL meal supplemented with baker's yeast as a supplement or total replacement of fishmeal for aquaculture industries, thus offering prospects towards more sustainable aquaculture practices. Further studies should develop a more nuanced understanding of the impact of yeast on specific bacterial communities, incorporating detailed pathological assessments to better understand the causes of mortality observed. It would also be interesting to use both defatted and non-defatted BSFL with baker's yeast and especially to analyse the nutritional quality of the fish.

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NOVELTY STATEMENT

This study presents a novel method for replacing fishmeal entirely in Nile tilapia diets: black soldier fly larvae (BSFL) meal supplemented with baker's yeast (*Saccharomyces cerevisiae).* This combination offers a sustainable, affordable alternative for aquaculture feed that tackles the issues of fishmeal dependency by utilizing the high protein content of BSFL with the probiotic advantages of baker's yeast to improve growth, feed efficiency, and gut health.

AUTHOR'S CONTRIBUTIONS

R. Ntakirutimana: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Software, Validation, Writing – review and editing.
KMM Rahiman: Conceptualization, Supervision, Methodology, Validation, Visualization, Writing – review and editing.
M. Lovejan: Data curation, Visualization, Formal analysis, Validation, Writing – review and editing.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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