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Effects of the Substitution of Fish Meal by a Combination of Black Soldier fly meal (*Hermitia illucens*) and Shea Butter Meal (*Cirina butyrospermi*) on the Growth Performance and Biochemical Composition of the Flesh of Nile tilapia *Oreochromis niloticus* Fry

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Abstract

Fishmeal is an essential component in fish diet. It can be replaced by different sources of protein to make fish feeding more inexpensive. In the present trial, fishmeal was substituted by a combination of black soldier fly (BSF) (*Hermitia illucens*) and shea caterpillar (*Cirina butyrospermi*) maggots in tilapia feed at different proportions to evaluate the growth performance and flesh biochemical composition of Nile tilapia *Oreochromis niloticus* fry. A total of one hundred and eighty (180) tilapia fry were placed in 9 x 80-liter fiberglass tanks at a density of 20 individuals per tank. Three diets were formulated, a control (R0) (based on fish meal), R1 diet (50% fish meal, 25% BSF and 25% caterpillar meal) and R2 diet (50% BSF and 50% caterpillar meal). The results of this study showed that the substitution of 50% fish meal by BSF meal and caterpillar meal had a positive effect on fish growth performance. Mean final weight (0.29 ± 0.04g) and relative mean weight gain (462.09 ± 74.13%) were more significant in fish nursed the R1 diet and less significant in fish fed the R2 diet, which were 0.19 ± 0.04g and 332.20 ± 135.55% respectively. There was no significant difference in the FCR obtained, but the lowest FCR was recorded with diet R1 at 2.69 ± 0.43. The specific growth rate (SGR) showed significant difference between the different experimental diets, with the best SGR obtained with diet R1 at 4.09 ± 0.43%/d. In addition, the biochemical composition of fry flesh revealed adequate levels of protein and lipids, suggesting a nutritional quality comparable to that of fry fed fishmeal alone. These findings indicate that the mixture of black soldier fly meal (25%) and shea caterpillar meal (25%) may be a promising alternative to fish meal in the feeding of Nile tilapia fry, offering similar growth performance and satisfactory flesh biochemical composition.

Keywords: black soldier fly meal, caterpillar meal, *Oreochromis niloticus*, growth

1. Introduction

Fish farming will provide almost two-thirds of the world's fish production for food by 2030, given the stabilization of wild fish catches and the growing demand from an emerging global middle class (FAO, 2019). Nile tilapia (*Oreochromis niloticus*) is a popular fish today for fish farming, aquaristics and aquaponics. It is the second most farmed fish in the world after carp, so that it is sometimes nicknamed the "Asian chicken". Its production doubled between 2005 and 2016, reaching 5.37 million tons (Deluzarche, 2021).

Global tilapia production has boomed, increasing 15-fold in thirty years. Global aquaculture production of tilapia rose from 380,000 tons in 1990 to 7 million tons in 2024 (FAO, 2024). The main producers of farmed tilapia are Egypt, Indonesia, China and Brazil. The two leading countries, Egypt and Indonesia, each produced almost 1.6 million tons in 2023. Other African tilapia producers, notably Uganda, Ghana and Zambia, are also showing strong growth. Through the contribution of these countries, Africa accounts for 27% of world tilapia production, with around 1,894,000 tons in 2023 (FAO, 2024).

Tilapia, due to their high adaptability to a wide range of physical and environmental conditions, ability to reproduce, relative resistance to stress and pathogens compared to other farmed fish species, good flesh quality and excellent growth rate on a wide variety of natural and artificial diets are the most abundantly cultivated species in the world. They are currently cultivated in virtually all types of production systems, in fresh and salt water and in tropical, subtropical and temperate climates (Welker and Lim, 2011). Taking into account its interesting characteristics in aquaculture and its good adaptation to extreme and varied environments, its ease of reproduction in captivitý and its wide ecological valence, this fish has been the subject of various breeding trials and is currently widely distributed in all continents since the 1960s (Azaza, 2009).

Fishmeal (FM) is one of the most important protein sources in fish feeds, particularly for carnivorous fish and crustaceans (Hua *et al*., 2019). Its richness in amino acids, good digestibility and palatability help improve feed intake, digestion and nutrient absorption in fish (Miles and Chapman, 2006). However, due to the depletion of wild fish stocks for fishmeal and fish oil production, these ingredients are no longer sustainable (FAO, 2018, Arru *et al*., 2019). To preserve natural resources and make aquaculture sustainable, it is imperative to reduce the use of ingredients derived from capture fisheries. The search for suitable and sustainable alternatives to fishmeal and fish oil is the subject of several studies, with potential ingredients coming from terrestrial plants, animal by-products, algae or insects (Gatlin *et al*., 2007; Olsen and Hasan 2012; Boyd and McNevin, 2015).

Insects are a valuable and sustainable source of protein. At present, a few insect species have been proposed for feeding to farmed fish, such as the milling sandfly (*Tenebrio molitor*) (Rema et al., 2019) as well as cricket meal (Ndione *et al*., 2022a), shea caterpillar (Ndione *et al.*, 2022b), and black soldier fly (Van Huis, 2013). Insects meals such as black soldier fly meal and shea caterpillar meal have nutritional values almost similar to fish meals in terms of their protein and essential amino acid composition. Insects are the most diverse group of animals and a natural food source for fish, particularly carnivorous and omnivorous fish, as these fish species require relatively high protein in their diet (Van Huis, 2020, Nogales-Merida *et al.*, 2019; Tran *et al.*, 2015).

The aim of this study is therefore to examine the effects of partial or total substitution of fish meal by a combination of insect meals (shea caterpillar and desert locust) on the growing performance of Nile tilapia (*Oreochromis niloticus*).

2.Materials and Methods

2.1 Feed Formulation

Three experimental diets R0, R1 and R2 were formulated using the Pearson square method (Table 1). In these diets, fish meal was replaced at 50% and 100% by a combination of black soldier fly meal and caterpillar meal. Thus R0 (control) contains no black soldier fly meal and no caterpillar meal, R1 contains a 50% mixture of black soldier fly meal and caterpillar meal and R2 contains 100% of the mixture of black soldier fly meal and caterpillar meal. Other ingredients such as maize meal, peanut cake meal, peanut pulp meal, *Neocarya* pulp meal (as binder) and rice polish meal were also used in the feeds. Feed containing 32% crude protein and 15% fat has also been enriched with vitamins and minerals.

Table 1. Centesimal composition of various diets

Ingredients	Control (R0)	R1 (50%)	R2 (100%)
Fish meal	30	15	0
Black soldier fly meal	0	7.5	15
Caterpillar meal	0	7.5	15
Maize meal	14	12	13
Peanut cake meal	8	14	20
Peanut pulp meal	12	10	7
Neocarya pulp meal	6	6	8
Rice polish meal	12	14	8
Brewery waste meal	8	5	5
Neocarya pulp meal	6	5	5
Fish oil	2	2	2
Vit[a]	1	1	1
Min[b]	1	1	1
Total (%)	100	100	100
Protein level (%)	30.96	30.61	30.38
Lipid level (%)	12.50	12.87	12.89

[a]=vit A 250000 UI; vit D3 250000UI; vit E 5000mg; vit B1 100mg; vit B2 400mg; vit B3(pp) 1000mg; vit B5 pantode Ca2000mg; vit B6 300mg; vit K3 1000g; vit C 5000mg; H biotin 15mg; choline 100g; anti-oxydant (BHT), crushed and calcined attapulgite qs 1000mg;

[b]=phosphorus 7%; calcium 17%; sodium 1,5%; potassium 4,6%; magnesium 7,5%; manganese 738mg; zinc 3000mg; iron 4000mg; copper 750mg; iodine 5mg; cobalt 208mg; calcined and ground attapulgite qs 1000g; fluorine 1.5% (approximately).

2.2 Diet Processing

Three diets, isoproteic (30% crude protein) and isolipidic (12% fat), were produced. Variable amounts of fish meal were replaced at 0% (R0), 50% (R1) and 100% (R2) by the combination of black soldier fly meal and shea caterpillar meal. Fish oil was used as a lipid source. Ingredients (Table 1) obtained from the local market were initially ground into small particles using a hammer mill, then passed through a 250 µm mesh sieve. After achieving homogeneity by mixing all the dry ingredients, fish oil was added. Next, water (around 30% of dry weight) was added to create a wet paste. This paste was then extruded through a 3.0 mm diameter die to produce pellets. The pellets were dried in a dry air oven at 60°C and then stored at -20°C.

2.3 Rearing Conditions

A study lasting 45 days was carried out at the Pr Omar Thiom Thiaw aquaculture station of the University Institute of Fishery and Aquaculture at Cheikh Anta DIOP University of Dakar. The tilapia fry used came from the aquaculture station. A total of 180 fingerlings were distributed in 9 fiberglass tanks of 80 liters with a density of 20 individuals/tank. Preceding to experimentation, the Nile tilapia fry were acclimatized for one week and fed a control diet twice a day at 9 am and 5 pm. The fish were fed these diets at 15% and 10% of their body weight respectively. Physico-chemical parameters were measured twice a day (8 am and 4:30 pm). Remaining diets and feces were removed by siphoning. Fry body weight (g) was evaluated every fifteen days during the trial period.

2.4 Zootechnical Parameters

To evaluate fish growth over the course of the experiment and characterize the utilization efficiency of the feeds tested, the following zootechnical parameters were calculated:

- Absolute Mean Weight Gain (AMWG) (g) = Final Mean Weight (FMW) (g) - Initial Mean Weight (IMW) (g)

- Relative Mean Weight Gain (RMWG) (%) = (Final Mean Weight (g) - Initial Mean Weight (g)) *100/ Initial Mean Weight (g)

- Specific Growth Rate (SGR) (%/day) = [ln (final weight) - ln (initial weight)] x 100 / Period

- Feed Conversion Ratio (FCR)= Dry feed intake / Body weight gain

- Survival Rate (SR) (%) = Final number of fish x 100 / Initial number of fish.

2.5 Analysis of Fish Flesh

At the end of the experiment, fish samples per tank from each diet were analyzed at the ENSA bromatology laboratory in Thiès, Senegal, using the AOAC (1995) method. Samples were dried at a constant temperature of 105°C to determine moisture content. Crude protein (calculated as total nitrogen multiplied by 6.25) was determined using the micro-kieldahl method (kjeltec system 1002 distillation unit from Tecator in Hoeganes, Sweden). Crude fat content was determined gravimetrically using a Soxhlet apparatus, and ash content was determined by subjecting samples to incineration in a bellows furnace at 550°C for 6 hours.

2.6 Statistical Analysis

The differences in Final Mean Weight (FMW), Absolute Mean Weight Gain (AMWG), Percentage Mean Weight Gain (RMWG), Specific Growth rate (SGR) and Feed Conversion ratio (FCR) between the three diets were assessed using one-way analysis of variance (ANOVA). Duncan's test was used to compare differences between diets. Significance was set at $P < 0.05$.

3. Results and Discussion

3.1 Results

3.1.1 Physico-Chemical Parameters

Water quality parameters were documented daily during the trial. The mean, minimum and maximum values per treatment are shown in Table 2.

Table 2. Physico-chemical parameters

Temperature (℃)	R0	R1	R2
Mean	26.31	26.23	26.27
Max	27.23	27.07	27.47
Min	25.07	25.15	25.24
pH			
Mean	8.59	8.56	8.55
Max	8.76	8.73	8.72
Min	8.8	8.44	8.18

The extreme values of temperatures at the different regimes range from 25.07 to 27.47℃ with an average of 26.27 ℃.

The pH values recorded in the different diets during the study period ranged from 8.18 to 8.76, with an average of 8.57.

3.1.2 Growth Parameters

Various growth parameters were studied during the experimental period and the results obtained are shown in Table 3.

Table 3. Fish growth parameters

Parameters	R0 (0% insects meal)	R1 (50% insects meal)	R2 (100% insects meal)
IMW (g)	0.06^{a}	0.06^{a}	0.06^{a}
FMW(g)	0.30 ± 0.03^{a}	0.35 ± 0.04^{a}	0.25 ± 0.03^{b}
AMWG (g)	0.25 ± 0.03^{a}	0.29 ± 0.04^{a}	0.19 ± 0.04^{b}
RMWG (%)	419.26 ± 76.02^{a}	462.09 ± 74.13^{a}	332.20 ± 135.55^{b}
SGR (%/day)	3.90 ± 0.37^{a}	4.09 ± 0.43^{a}	3.37 ± 0.31^{b}
FCR	2.95 ± 0.11^{b}	2.69 ± 0.43^{a}	2.95 ± 1.36^{b}
SR (%)	76.67 ± 2.87^{a}	81.67 ± 10.40^{a}	70 ± 18.02^{a}

The differences in letters (a and b) in the superscript indicate a significant difference ($P<0.05$).

Fish fed R1 did not show any significant difference with those fed the control diet (R0).

However, fish fed diet R2 show significant difference with the fish fed the control diet (R0). Fish fed the R1 diet showed significantly ($P < 0.05$) faster growth than those fed R2 diet. With an average weight gain of 0.29 g over 45 days and a specific growth rate of 4.09%/day, fish fed the R1 diet showed the best growth performance compared with the other two diets. Statistically, these results were not different from those obtained with the control diet. At the end of the experiment, taking into account the biomass produced and the total amount of feed distributed, the feed conversion ratio (FCR) of each diet was calculated. It ranged from 2.69 ± 0.43 for diet R1 to 2.95 ± 0.11 and 2.95 ± 1.36 for diets R0 and R2 respectively. Diet R1 performed better than diet R0 (control) and R2. Over the whole duration of the experiment, the survival rate in the different diets was 76.67%, 81.67% and 70% respectively for diets R0, R1 and R2. No significant difference between these survivals mean values was observed.

3.1.3 Biochemical Composition of Fish Flesh

The results of fish flesh analyses are presented in Table 4.

Table 4. Biochemical composition of fish flesh

Parameters	R0 (0% insects meal)	R1 (50% insects meal)	R2 (100% insects meal)
Ash (% DM)	4.64 ± 1.22[a]	3.86 ± 0,66[a]	3.35 ± 1.11[a]
Crude Protein (% DM)	14.62 ± 1.64[a]	14.09 ± 1.63[a]	14.66 ± 0.64[a]
Crude Fat (% DM)	4.65 ± 1.33[a]	3.98 ± 0.76[a]	4.70 ± 1.3[a]

Letter (a) in the superscript indicated no significant difference ($P > 0.05$).

The results obtained for the biochemical composition of the flesh show that no significant statistical differences were observed for protein, fat and ash levels. Value wise, Ash levels were higher in fish fed the R0 control diet with 4.64% DM. With regard to protein levels, the results show that fish fed the different diets are rich in protein respectively for diets R0, R1 and R2. Fish fed the R2 diet had the highest protein value content.

3.2 Discussion

3.2.1 Physico-Chemical Parameters

The physico-chemical parameters monitored are of major importance in the management of fish farming, as inadequate values of these parameters can influence the physiological state of the fish.

In this study, the temperature values in the tanks did not vary significantly. At diet level, they ranged from 25.07 to 27.47 °C. This value falls within the range recommended for Tilapia farming by Suresh (2003), who limits this parameter to 26-32°C. In the natural environment, Tilapia is a eurythmic fish that can withstand large variations in water temperature (CTA, 2017).

The pH represents the acidity or alkalinity of the water. Throughout the experiment, values ranged from 8.18 to 8.76. These values do not fall within the optimal range recommended (Malcolm *et al.*, 2000), which is between 6.5 and 8.5. Abdullah *et al.* (2017) reported that alkaline water (pH 8.5 - 10) causes acute physiological disturbances in fish, affecting normal growth rates and ultimately becoming a potentially lethal factor for fish. This could explain the mortalities observed in the present study.

3.2.2 Parameters for Growth, Feed Efficiency and Biochemical Composition

The fast development of farming industry has put pressure on the supply of fish meal, which is derived from pelagic fish species. It has therefore become essential to substitute fish meal with various sources of proteins such as animal proteins (insect meal, poultry co-products, blood meal etc.) to produce high-quality fish, minimize feed costs and overfishing of pelagic fish for fishmeal production (Mmanda *et al.*, 2020). Between animal proteins, insects are the most promising and appropriate substitutes for fish meal (Gasco *et al.*, 2020). In this study, the substitution of fish meal by black soldier fly meal and shea caterpillar meal had a positive effect on fish growth performance. The highest average final mean weight, absolute mean weight gain and specific growth rate were recorded in fish fed the R1 diet with (0.35g; 0.29g and 4.09%/day) respectively. However, non-significant differences were observed between fish fed the R1 diet and those fed the R0 control diet. These values do not agree with those obtained by Ndione *et al.* (2022b), who obtained a final average weight, absolute mean weight gain and specific growth rate of (1.78g; 1.56g and 3.7%/day) respectively in diet (containing 50% caterpillar meal) in their study of the effects of partial or total substitution of fish meal by shea caterpillar meal (*Cirina Butyrospermi*) on Nile tilapia.

In the present study, fish fed the R1 diet achieved the lowest FCR (2.69), which showed no significant difference from the FCR of the R0 and R2 diets (2.95). These results are not in line with those of Limbu *et al.* (2022) who obtained the lowest FCR (0.89) with tilapia fry fed with up to 75% black soldier fly meal. This value is also inconsistent with that reported by Ndione *et al.* (2022b), who obtained 1.50 with diet (containing 50% caterpillar meal) in their study on the effects of partial or total substitution of fish meal by caterpillar meal on Nile tilapia. The reduction in fish growth performance and feed efficiency with increasing levels of substitution (up to 100%) in this study could be owing to the presence of chitin in black soldier fly meal. Eggink *et al*. (2022) indicated that rainbow trout and Nile tilapia have the capability to digest chitin, but chitin digestibility decreases with higher chitin inclusion, confirming that chitin could act as an anti-nutritional factor in both fish species.

3.2.3 Biochemical Composition of Fish Flesh

The biochemical composition of fish flesh using a combination of black soldier fly meal and caterpillar meal showed no significant difference between the different diets**.**

The protein content ranged from 14.09 to 14.66%. These results do not agree with those of Ndione *et al.* (2022b), who obtained protein levels ranging from 65.91 and 66.92% in tilapia fry fed the diets substituted with contained 50 % and 25% of Caterpillar meal, respectively. These differences may be due the nature of the samples, wet base in the present study and dry base in the experiment of Ndione *et al.* (2022b). On the other hand, they are close to those of Yildirim *et al.* (2020), who obtained 15.06 and 15.21% in hybrids of (*Oreochromis niloticus* x *Oreochromis mossambicus*) fed diets with the highest inclusion rates of 20 and 30% black soldier fly frass.

In the present study, the lipid content of the fish ranged from 3.98 to 4.70%. The lowest value was obtained in the Diet R1 containing 50% of the combination of black soldier flies and caterpillar meal. The lipid content of the soldier fly is generally lauric acid, which is stored less in the fish body due to its quick oxidation (Liland *et al.*, 2017) and this could be the reason for the reduced fat content in Nile tilapia. These values are lower than those obtained by Yildirim *et al.* (2020), who found 5.84 and 5.88% in the flesh of hybrids of (*Oreochromis niloticus* x *O. mossambicus*) fed with 20% and 30% black soldier fly frass. The results of the present study agree with those of Devic *et al.* (2018), who reported that the level of inclusion of black soldier fly meal (up to 80%) had no effect on the proximal body composition of Nile tilapia. There are various factors responsible for variation in proximate fish composition. These involve differences between fish species, growth performance, and rates of protein and fat synthesis and deposition (Abdel-Tawwab *et al*., 2006).

4. Conclusion

Aquaculture, now represents the fastest-growing food production sub-sector in many countries (FAO, 2018). In current years, the overall supply of food fish has grown at a rate greater than global population growth, and fish is now a significant source of nutritious foodstuff and animal protein for a large proportion of the world's population. To this end, improving fish farming while preserving our environment has become a global concern. The aim of this study was to evaluate the effects of partial or total substitution of fish meal by a combination of black soldier fly meal and shea caterpillar meal.

It was observed that the replacement of 50% fish meal by a mixture of 25% BSF meal and 25% caterpillar meal in the diet gave satisfactory results in terms of growth performance evaluation, even though flesh analysis results showed no significant difference in Nile tilapia (*Oreochromis niloticus*) fry. Survival rates were also satisfactory (70 to 81.67%) despite the mortalities observed during the experimental period.

However, further research is needed on the impact of feeding insect meal to aquaculture species on safety, quality and societal acceptance, the effects of insect meal on the immune response of fish.

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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