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Special Graduation Project

**Assessing the viability of Black Soldier Fly larvae (*Hermetia illucens*) as
an alternative fish feed in rearing fry of Nile tilapia (*Oreochromis
niloticus*)**

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Summary

Alternative feeds, such as black soldier fly (*Hermetia illucens*) larvae, are emerging as sustainable animal feed alternatives due to their efficient feed conversion, low resource requirements, and high nutrient content. Therefore, the objective of this study is to evaluate the inclusion of black soldier fly larvae on the performance of Nile tilapia fry in comparison to a conventional feed. Over 42 days a total of 600 tilapia fry with an average weight of 0.4 g were sourced after the sex reversion stage and distributed amongst 12 tanks in groups of 50. The study included a control diet and three experimental diets with larvae meal replacing 25%, 50%, and 75% of the conventional protein source. Samples were collected from each tank every seven days to assess the weight gain of the tilapia fry. A completely randomized experiment was done along with an ANOVA and Duncan mean test. The data analysis on the final mean weight and daily weight gain showed that the differences between the experimental diets were negligible ($P > 0.10$). However, there was a significant difference ($P < 0.10$) between the experimental diets and the control diet. There were no significant differences in specific growth rate, survival, and feed conversion ratio ($P > 0.10$). The results suggest that black soldier fly larvae meal can serve as an alternative protein source. Full conversion from conventional feed to fly larvae meal could enhance environmental sustainability, but further research is needed to evaluate the environmental impacts of larvae production and other alternatives.

Keywords: Aquaculture, growth parameters, sustainability

Resumen

Los ingredientes alternativos, como los insectos, en particular las larvas de la mosca soldado (*Hermetia illucens*), se perfilan como alternativas sostenibles de alimentación animal debido a su eficiente conversión alimenticia, sus bajos requerimientos de recursos y su alto contenido en nutrientes. Por tanto, el objetivo de este estudio fue evaluar la inclusión de larvas de mosca soldado en el rendimiento de alevines de tilapia del Nilo en comparación con un alimento convencional. El experimento tuvo una duración de 42 días. Un total de 600 alevines de tilapia, con un peso medio de 0.4 g, se obtuvieron tras la fase de reversión sexual y se distribuyeron en 12 tanques en grupos de 50. Los tratamientos consistieron en una dieta de control y dietas con larvas de mosca soldado, siendo estas dietas experimentales consistentes en la sustitución del 25, 50 y 75% de la fuente proteica convencional por harina de larvas. Se tomaron muestras cada siete días de cada tanque para evaluar el aumento de peso de los alevines de tilapia. Se realizó un experimento completamente aleatorizado junto con un análisis de variancia (ANOVA) y una prueba de medias de Duncan. El análisis de los datos sobre el peso medio final y la ganancia diaria de peso mostró que las diferencias entre las dietas experimentales eran mínimas ($P > 0.10$); hubo una diferencia significativa ($P < 0.10$) entre las dietas experimentales y la dieta de control. No hubo diferencias significativas en la tasa de crecimiento específico, la supervivencia y la conversión alimenticia ($P > 0.10$). Los resultados sugieren que la harina de larvas de mosca soldado negra puede servir como una fuente alternativa de proteína.

Palabras clave: Acuicultura, parámetros de crecimiento, sostenibilidad

Introduction

The current world population is expected to reach 9.7 billion in 2050 (United Nations, n.d.). Similarly, the demand for food will rise by 56% (van Dijk et al., 2021). Therefore, agricultural industries such as aquaculture will play a key role in providing a sustainable source of protein. According to a 2020 report by the Food and Agriculture Organization of the United Nations (FAO) (Geehan et al., 2020), global fish consumption increased from 0.90 kg per capita in 1961 to 20.5 kg per capita in 2018. Additionally, global fish production in 2018 was approximately 179 million tons of which 82 tons derived from aquaculture production.

Despite continuous innovations within the industry, it is not without its faults. To supply the demand for aquaculture products, specially formulated diets are essential for meeting the nutritional requirements of each species to be cultivated. In the case of fish farming, fish feed must be a source that provides sufficient amino acids, lipids, carbohydrates, and minerals (Craig y Helfrich, 2017). Important amino acids are usually supplied using fish oil or fish-based feeds (Turchini et al., 2010). In 2018 it was reported that 18 million tons of fish were processed for fish oil extraction (Geehan et al., 2020). However, due to increasing global demand, wild fish stocks are declining (Sumaila y Tai, 2020) threatening the health of marine ecosystems and resulting in price inflation of fish oil/feed, therefore, worsening the cost of production. By 2030, the price of fishmeal and fish oil are expected to increase by 30% resulting in a 22% increase in the price of internationally traded fish (Geehan et al., 2020).

To lower the cost of production, much research and development has been invested in formulating alternative feeds. Given the importance of fish feed supplying quality sources of protein, lipids and carbohydrates, some substitutes that are being investigated include plant-based diets (Bandara, 2018). Common plant-based sources of protein include soybean meal, guar meal, corn gluten, wheat gluten, peas, byproducts of sugar cane, potato, canola, and cassava (Hodar et al., 2019). Carbohydrate ingredients include corn, wheat, rice, and potato starch. As for lipids/fats, they're sourced from vegetable oil, soybean oil, rapeseed oil, and sunflower oil (Nagappan et al., 2021).

However, the use of plant-based feeds has presented its own challenges by having reduced palatability, digestive issues, and deficiencies in certain amino acids (Bandara, 2018).

Therefore, alternative sources such as insects are now being considered as a potentially reliable and sustainable animal feed due to their efficient feed conversion ratio and high nutrient content. According to Baiano, 2020, to produce 1 kg of edible protein, insects only require 10% of the land needed for beef production. Additional studies have also found that various insects may contain protein levels of 13 to 77% of their dry biomass (Jonas-Levi y Martinez, 2017). Such insects include the black soldier fly (Insecta: Diptera: Stratiomyidae: Hermetiinae: *Hermetia illucens*). It is characterized as a long, three-segment fly with brownish wings. In its larval stage, it contains 18 to 33% fat and 32 to 53% protein depending on the quality of its own food. Black soldier fly larvae are also known for having a rich amino acid and fatty acid profile comprising of leucine, lysine, valine, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and palmitoleic acid (Lu et al., 2022). Therefore, black soldier fly larvae offer the possibility of completely replacing fish meal as a sustainable alternative feed.

Recent studies have shown that black soldier fly larvae can successfully be used in the diets of multiple fish species. For instance, a study conducted using rainbow trout showed that black soldier fly larvae meal could replace 40% of their traditional diet, producing similar growth parameters to that of fish meal (Renna et al., 2017). Similar results were seen in Atlantic Salmon (Belghit et al., 2019), Siberian Sturgeon (Caimi et al., 2020), and Nile Tilapia in the fry stage of production (Limbu et al., 2022).

The Nile tilapia is recognized as the most popularly farmed fish worldwide with significant economic importance (Munguti et al., 2022). The fry stage of production is crucial for maintaining a continuous product supply. Considering the need to evaluate the effectiveness of alternative fish feeds such as insects in different production stages to improve the sustainability of the aquaculture industry, the purpose of this study is to evaluate the weight gain of Nile tilapia fry fed with black soldier

fly larvae meal. To complete this study, the following objectives have been outlined: a) Assess the efficiency of black soldier fly larvae feed on the growth parameters of Nile tilapia fry; and b) Compare the performance of black soldier fly larvae feed to conventional feed on the growth parameters of Nile tilapia fry.

Methodology

Research Area

The study was conducted at the Monte Redondo aquaculture facility within the Pan-American Agricultural School, Zamorano, 30 km from Tegucigalpa, Honduras. The facility is positioned approximately 800 m above sea level, it has a tropical dry climate with an average annual temperature of 24 - 25 °C and an average annual rainfall of 1,100 mm (Hernández, 2021).

The aquaculture unit is dedicated to producing tilapia by managing all the following stages of production: reproduction, fattening, and processing. The fish are raised within open ponds with water sourced from the reservoir at Monte Redondo and are fed specially formulated feed according to their stage of maturity.

Experimental Design

Treatments

A total of 600 tilapia fry (*Oreochromis niloticus*) weighing approximately 0.4 g each were sourced from the aquaculture unit. They were then distributed amongst 12 fiberglass tanks with a volume of 200 L measuring 1.0 m in diameter and 0.4 m in height. The tanks were placed within the greenhouse to avoid predation and to maintain a controlled water temperature. The fry were transferred to the greenhouse after the sexual reversion process and divided into groups of 50 where they were kept for the duration of the experiment.

The experiment included four treatments with three repetitions for each treatment, giving a total of 12 experimental units. The experimental units consisted of one control and three experimental diets all of which were formulated and mixed by hand. The control diet was formulated using beef meat flour which has a total 53% protein (Table 1) that was sourced from an external provider.

Table 1*Nutritional content of meat meal containing 53% protein*

Parameters	Content (g/100 g)
Humidity	4.02
Dry matter	95.98
Protein	52.9
Protein nitrogen	8.30
Fiber	1.54
Ether extract	14.07

Note. Laboratorio de Control de Calidad San Jose

As for the experimental diets, each contained varying percentages of black soldier fly larvae meal to replace the conventional feed of meat flour. The percentages used were 25, 50, and 75% inclusion of larvae meal. To prepare different diets, the larvae were first sourced from the Zamorano's Entomology laboratory. They were then dried for 4 - 5 days and finely ground using a meat grinder. The experimental diets were then formulated and mixed by hand using the established inclusion percentages of the larvae meal, along with additional ingredients to meet the nutritional requirements of the tilapia fry. The nutrient content of the larvae which has a total of 40% protein is shown in Table 2. It should be noted that the chemical proximate analysis that was used as a reference had analyzed black soldier fly larvae from a previous study which were produced using a similar diet of food waste from the cafeteria. The formulation of both the control diet containing meat meal and the experimental diets containing larval meal, along with the six additional ingredients are shown in Table 3.

Table 2*Chemical proximate analysis of *Hermetia illucens**

Parameter	Units	Concentration
Ashes	g/100 g	4.36
Protein	g/100 g	40.58
Humidity	g/100 g	4.00
Fat	g/100 g	41.45
Raw fiber	g/100 g	9.61
Digestible Carbohydrates	g/100 g	0.00
Gross energy	kcal/kg	5,738.10

Note. Adapted from Laboratorio de Análisis de Alimentos Zamorano (2022)

Table 3*Experimental Treatment Feed Formulations*

Ingredients	Experimental Treatments			
	Control	HI 25%	HI 50%	HI 75%
Corn flour	16.35	19.55	14.87	8.37
Meat flour	30.00	17.50	5.00	--
Soy flour	45.50	37.00	29.00	14.00
<i>Hermetia illucens</i> larvae flour	--	25.00	50.00	75.00
Palm oil	7.50	0.30	--	--
Calcium carbonate	--	--	0.37	0.70
Biofos	--	--	0.11	0.78
Common salt	0.40	0.40	0.40	0.40
Broiler vitamin	0.25	0.25	0.25	0.25

Note. The values are expressed as lb/100 lb

The fish fry were fed six times a day; three times in the morning and three times in the afternoon. This was done every hour after the first round to ensure feed efficiency and reduce feed waste. Ideally, they would be fed a maximum of 13% of their body weight per day according to standard feeding charts. However, feeding the full standard amount depended solely on the appetite of the tilapia fry. This was determined by the amount of feed that remained on the water's surface and the behavior of the tilapia fry's during feeding period. Additionally, the tilapia fry were not fed at the time of a water change or sampling due to loss of appetite caused by stress.

Water Quality Parameters***Dissolved Oxygen***

It represents the concentration of gaseous oxygen dissolved in water expressed in mg/L. It is important in tilapia production as low levels of dissolved oxygen can cause slow growth, death, or increase disease susceptibility. The dissolved oxygen levels were measured twice a day, in the morning and afternoon using the YSI Pro20 sensor. The optimal range of dissolved oxygen is ≥ 3.0 mg/L (Abd El-Hack et al., 2022).

Water Temperature

Fish are poikilothermic animals, meaning that their body temperature depends on the temperature of their environment. Therefore, water temperature directly affects their metabolic system, determining how much they consume. The higher the temperature, the more fishmeal is consumed, and the lower the temperature the less. Preferable water temperature is between 28 - 32 °C (Abd El-Hack et al., 2022). This was measured twice a day in the morning and afternoon using the YSI Pro20 sensor.

Nitrate

This parameter represents the amount of nitrate ion (NO_3^-) and is expressed in mg/L. High levels of nitrate oxidize hemoglobin, reducing its oxygen carrying capacity which affects the growth rate and mortality of tilapia. Nitrate levels should remain within the range of 1.5 - 2.0 mg/L (Shokr, 2015). This was measured twice a week using the API test kit. To do so, the nitrate test consists of filling a clean test tube with 5 ml of water, adding ten drops of bottle No. 1 of nitrate (NO_3^-), shaking the tube several times in order to mix the solution, then adding ten drops of bottle No. 2 of nitrate (NO_3^-), and shaking vigorously for 1 minute, waiting five minutes for the color to develop and finally comparing the color of the solution with the nitrate chart.

Nitrite

Nitrite (NO_2^-) is a form of nitrogen that is produced when ammonia is converted to nitrate and is expressed in mg/L. High nitrite levels oxidize hemoglobin, reducing its oxygen carrying capacity which affects the growth rate and mortality of tilapia. Acceptable nitrite levels are ≤ 0.1 mg/L (Yanbo et al., 2006) and were measured twice a week using the API test kit. Measuring nitrite levels consists of filling a clean test tube with 5 ml of water, adding five drops of the nitrite (NO_2^-) bottle, shake the tube for five seconds, wait 5 minutes for the color to develop and finally compare the color of the solution with the nitrite color chart.

Ammonia

Ammonia levels indicate the concentration of two nitrogen compounds, unionized ammonia (NH_3) and ammonium ion (NH_4^+) which are produced from the decomposition of organic matter. High ammonia levels can cause tilapia to lose appetite, reduce growth rate or die. Acceptable ammonia levels are ≤ 0.1 mg/L (Abd El-Hack et al., 2022) and were measured twice a week using the API test kit. The ammonia test consists of filling a clean test tube with 5 ml of water, adding eight drops of the two ammonium solutions ($\text{NH}_3/\text{NH}_4^+$), shaking vigorously for 5 seconds, waiting five minutes for the color to develop, and finally comparing the color of the solution with the ammonium color chart.

pH

The pH levels must be between the range of 6.5–9 and were measured twice a week using the API test kit. Drastic changes in pH can cause stress and significantly reduce the growth rate of tilapia (Abd El-Hack et al., 2022). The pH test involves filling a clean test tube with 5 mL of water, adding three drops of the pH solution, shaking the tube several times to mix the solution and reading the result by comparing the color of the solution with the pH chart.

Growth Parameters

Sampling

Fish growth was evaluated every seven days requiring that 15 tilapia fry were randomly selected and weighed in groups of five. This was repeated for each of the 12 tanks. Once the final data had been collected the following parameters were analyzed:

Final Mean Weight

It represents the body weight of the animals in the experimental units at the end of the study.

This is calculated using Equation 1.

$$\text{Final Mean Weight} = \frac{\text{Biomass (g)}}{\text{Number of animals}} \quad [1]$$

Feed Conversion Ratio

This variable provides information on the capacity of animals to convert feed into biomass during the period of their production cycle. This parameter is calculated using Equation 2.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Amount of food supplied (g)}}{\text{Final biomass (g)} - \text{Initial biomass (g)}} \quad [2]$$

Survival

This variable allows us to know the percentage of animals that survived during the time of the trial. It's calculated using Equation 3.

$$\text{Survival} = \frac{\text{Harvested fish}}{\text{Seeded fish}} \times 100 \quad [3]$$

Daily Weight Gain

Daily weight gain determines the average number of grams of weight gained by each animal per day throughout the trial. This is calculated by using Equation 4.

$$\text{Daily Weight Gain} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Age (days)}} \quad [4]$$

Statistical Analysis

A completely randomized correlational experimental design was used with three experimental diets and a control. The experiment was carried out for a period of 45 days using a 4 × 3 design, meaning four variables with three replicates. The growth parameters of each treatment were evaluated to determine the ideal percentage of larvae meal to be used. This information was used to evaluate the efficiency between conventional and unconventional fish feeds. For this purpose, the statistical method Analysis of Variance (ANOVA) ($\alpha = 0.1$) along with a Duncan's multiple range test was used to evaluate the differences between the means of the following variables: daily weight gain, feed conversion ratio, final weight, specific growth rate, and survival. The Statistical Analysis System (SAS) was used for this purpose. As for the water quality parameters, the minimum and maximum values were evaluated using Microsoft Excel.

Results and Discussion

Growth Parameters

The final mean weight differed significantly among the four treatments ($P < 0.10$; Table 4). However, a Duncan test revealed that the differences between the experimental diets (HI25, HI50, HI75) were negligible. The significant difference was between the experimental diets and the control. On average, the experimental diets presented the following increase in final mean weight with respect to the control: HI25- 29.55%, HI50- 32.95%, and HI75- 34.94%.

Table 4

The effects of Hermetia illucens meal on the growth parameters of Nile tilapia fry.

Treatment	F.M.W (g) Mean	D.W.G (g) Mean	Survival (%) Mean
Control	3.52	0.07	87
HI 25%	4.56 ^a	0.10 ^a	93 ^a
HI 50%	4.68 ^a	0.10 ^a	94 ^a
HI 75%	4.75 ^a	0.10 ^a	91 ^a
F value	2.64	2.77	1.86
Probability	0.065	0.059	0.14

Note. Values with ^a are not significantly different. F.M.W: Final mean weight. D.W.G: Daily weight gain. F value: The ratio of two variances.

The daily weight gain differed significantly among the four treatments ($P < 0.059$; Table 4). However, a Duncan test revealed that the differences between the experimental diets (HI25, HI50, HI75) were insignificant. On average, the experimental diets (HI25, HI50, HI75) presented 42.86% higher daily weight gain than the control.

The survival data showed no significant differences among the four treatments ($P > 0.10$; Table 4). These results are also supported by a Duncan's test. However, it appears that the control has the lowest survival rate compared to the other treatments. On average, the experimental treatments showed approximately 6% higher survival than the control.

Feed conversion ratio was calculated per week for each experimental diet (Table 5). These was then used to perform an ANOVA test which gave a p-value of 0.40 (> 0.10) indicating that there were no significant differences between the four treatments.

Table 5

The effects of Hermetia illucens on the feed conversion ratio of Nile tilapia fry.

Week	Control	HI 25%	HI 50%	HI 75%
	FCR	FCR	FCR	FCR
1	2.95 ^a	1.44 ^a	4.23 ^a	2.90 ^a
2	0.68 ^a	1.06 ^a	0.64 ^a	0.58 ^a
3	1.77 ^a	0.76 ^a	1.73 ^a	0.87 ^a
4	0.63 ^a	7.10 ^a	0.89 ^a	0.61 ^a
5	0.63 ^a	0.31 ^a	0.43 ^a	0.49 ^a
6	-5.23 ^a	0.84 ^a	1.97 ^a	0.64 ^a
F value	1.08	1.08	1.08	1.08
Probability	0.40	0.40	0.40	0.40

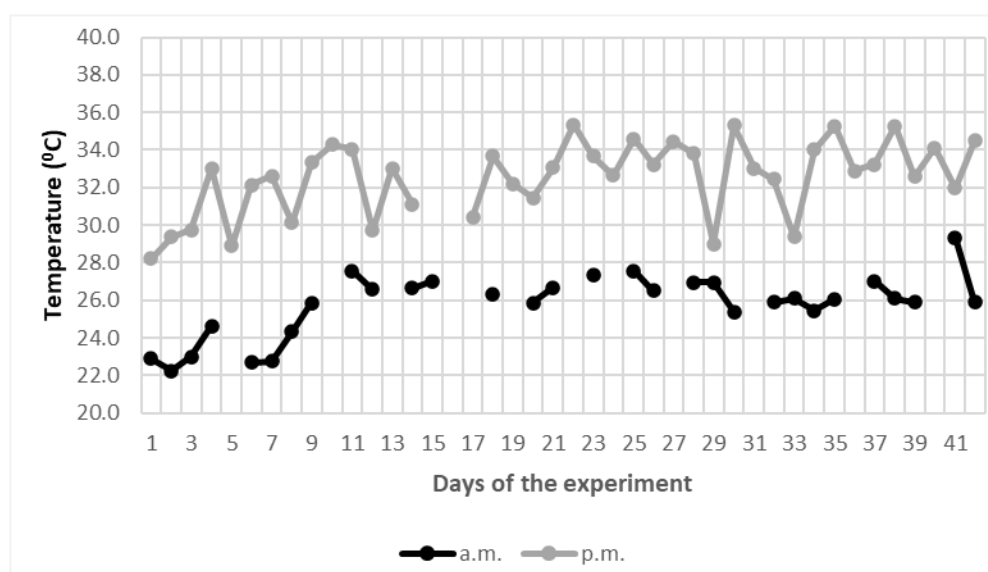
Note. FCR: Feed conversion ratio. F value: The ratio of two variances.

Water Quality Parameters

Water temperature was recorded both in the morning and in the afternoon throughout the experimental period (Figure 1). The average temperature recorded in the morning was 25.8 °C and 32.5 °C in the afternoon. Morning temperatures ranged from a minimum of 22.2 °C to maximum of 29.3 °C. Afternoon temperatures ranged from a low of 28.2 °C to a high of 35.4 °C. Temperatures also remained relatively the same for each tank during both halves of the day.

Figure 1

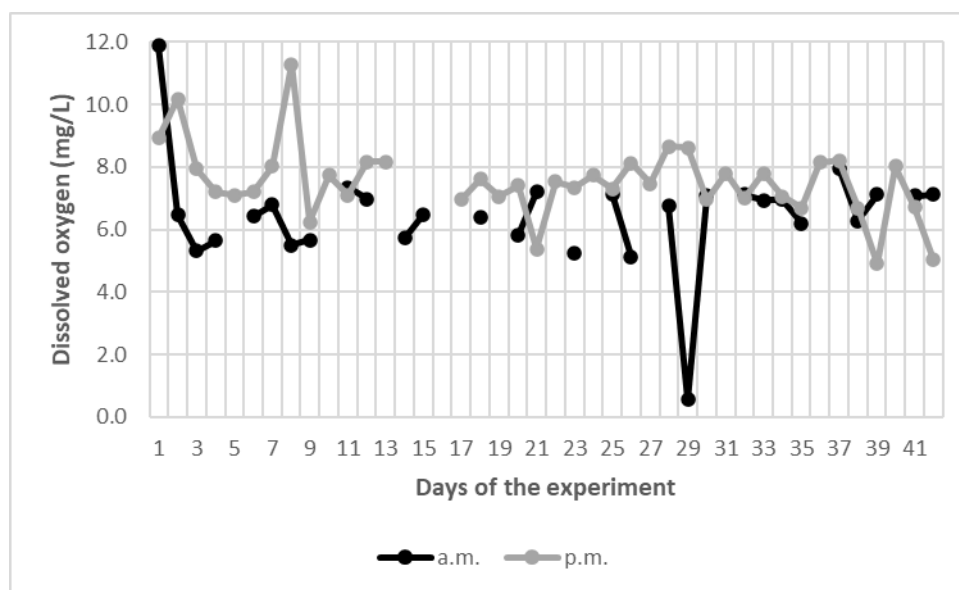
The temperature recorded over the period of the experiment



The dissolved oxygen was recorded both in the morning and afternoon throughout the experiment (Figure 2). The average dissolved oxygen was 6.5 mg/L in the morning (range: 0.6 to 11.9) and 7.5 mg/L in the afternoon (range 4.9 to 11.3).

Figure 2

The dissolved oxygen recorded over the period of the experiment



The average ammonia level was within 0.2 - 0.3 mg/L. The highest ammonia level recorded in various tanks throughout the study was 2 mg/L (Table 6); which was outside of the recommended range of ≤ 0.1 mg/L (Abd El-Hack et al., 2022). The average nitrate level was within 1.1 - 15 mg/L with the highest level recorded as 80 mg/L, which falls outside acceptable range of 1.5 - 2.0 mg/L. The average nitrite level was recorded as 0.0 - 5 mg/L with the maximum noted as 5 mg/L, surpassing the recommended range of ≤ 0.1 mg/L. The average pH levels recorded per treatment were Control- 7.9, HI25- 7.9, HI50- 7.9, and HI75- 7.8 (Table 6).

Table 6

Average and maximum values presented for each water quality parameter over a six-week period

Parameter	Control		HI 25%		HI 50%		HI 75%	
	Max	Average	Max	Average	Max	Average	Max	Average
Ammonia (mg/L)	0.5	0.2	1	0.3	1	0.3	2	0.2
Nitrate (mg/L)	10	1.1	80.0	15.0	80	13.9	80	8.0
Nitrite (mg/L)	0.25	0.0	5	0.7	5	0.6	2	0.3
pH	8.8	7.9	8.8	7.9	8.8	7.9	8.8	7.8

Note. mg/L: milligrams per liter. Max: Maximum.

It is important to note a few of the challenges that were experienced throughout the experiment. These may have altered the results in some form or another. The initial plan was to compare the experimental diets to fish meal (the control), however, we were unable to find a source. Therefore, meat meal was used as an alternative. Throughout the experiment students also assisted with routine activities, including feeding the tilapia in my absence. However, there were instances when students had not followed the proper guidelines, or no one was available to feed the tilapia. To measure the performance of the tilapia, samples were taken every seven days. As for the procedures regarding the sampling method, these were not followed for the first four weeks possibly affecting the representation of the population per tank.

The results of the final mean weight indicates that the inclusion of *Hermetia illucens* in the diet of tilapia fry can completely replace the protein source in conventional feed. This suggests that regardless of the portion, by incorporating larval meal it can improve weight gain. Therefore, it serves as an alternative feed, which supports the hypothesis of the experiment. In a similar study with tilapia fry, the authors found that diets containing 50 - 75% black soldier fly larvae meal also produced a higher mean final weight (Perez-Velazquez et al., 2024). It is important to note that the objectives of the study were different from the objectives of the present experiment. Perez-Velazquez et al. (2024) focused on evaluating the nutritional value of hydrolyzed and non-hydrolyzed black soldier fly larvae meal on Nile tilapia. Additionally, the protein content of the control diet consisted of multiple

ingredients such as fish meal, soybean meal, and corn meal. The study also used less and smaller tilapia fry (560 fry, $\bar{x}$ 0.26 g), but had a similar duration of 42 days.

The results of the daily weight gain further establishes that black soldier fly larvae meal can completely substitute conventional sources of protein, thus supporting the experiment's hypothesis. The outcome is consistent with the findings of Tippayadara et al., (2021) who concluded that there were no significant differences in the daily weight gain of tilapia fry fed diets containing between 10% to 100% black soldier fly larvae meal. As for the objectives of the study, they slightly varied as Tippayadara et al. (2021) also evaluated the effects of black soldier fly larvae meal on the hematology and skin mucus of Nile tilapia. The control utilized fish meal, soy meal and corn meal as its protein source. Moreover, larger and less tilapia fry (420 fry, $\bar{x}$ 14.77 g) were also used with the experiment lasting a total of 12 weeks; 6 more weeks than the present experiment.

There were no significant differences between the treatments, so it is impossible to say which diet had the best survival rate. Nonetheless, a possible explanation for an apparent lower survival rate in the control group is linked to nine deaths that were recorded within the first week. It is difficult to conclude that these deaths were related to water quality as the chemical parameters remained acceptable during the first week. Similarly, it is difficult to determine that the diet was the culprit, as no other deaths were recorded within the following weeks. An alternative theory could be that the deaths were possibly related to the stress of transferring the tilapia fry to the tanks. However, there is no definitive explanation, and the circumstances would require further investigation.

Considering the lack of significant differences among the experimental diets it cannot be said that one had the best feed conversion ratio (FCR). This suggests that both the larvae meal and meat flour are equally efficient in providing the necessary nutritional requirements without hindering the growth of the tilapia fry. The findings are supported by those of Tippayadara et al. (2021), who also found no significant differences between the control and experimental diets containing up to a 100% black soldier larvae meal for tilapia fry. Nevertheless, apparent differences can be found within a few

weeks with FCR values higher than two or lower than zero. According to Mengistu et al., (2020), an acceptable FCR is considered acceptable when it is two or less. This would mean that less feed was required to produce one gram of body weight, or that the fish used more of its energy reserves than what was consumed. Finally, it should be noted that the data for FCR were difficult to collect and the lack of differences may be due to errors in data collection.

Although the water quality parameters are essential to ensure a healthy environment for the development of the tilapia, it was not the focus of the study. The experiment was run to avoid variation in water quality parameters among treatments, although it is always possible that variation existed. Therefore, very general statistical analyses were done.

For the most part both the morning and afternoon temperatures were outside of the recommended range of 28 - 32 °C. According to Abd El-Hack et al. (2022), temperature is an important factor in tilapia production considering that they're poikilothermic, meaning their metabolism depends on the temperature of their environment. This means that warmer weather would prompt tilapia to consume more feed which is the goal of the producer. Similarly, during the experiment it was observed that the greatest feed consumption was recorded during the afternoon in comparison to the morning when it is colder. However, extreme temperatures can cause stress or increase the development of diseases. Meanwhile, low temperature decreases the metabolism of the fish causing them to consume less feed. Besides the ambient air temperature of the location, the fact that the tanks were kept within a greenhouse also contributes to the elevated water temperature. As a recommendation, it would be useful to install fans to create an adequate environment during such periods.

Total dissolved oxygen levels remained above the minimum acceptable level of ≥ 3 mg/L. At no time during the experiment did the dissolved oxygen levels fall below the recommended level (Abd El-Hack et al., 2022). However, oxygen levels varied within each tank. A possible cause could have been the pump or the air stone diffusing the air within the tank. Nonetheless, this would have required

further investigation to determine the exact cause. If dissolved oxygen levels were to drop dramatically it would reduce the fish's activity, reduce their consumption rates, or become fatal.

Throughout the study there were various occasions when the ammonia levels were outside of the recommended range of ≤ 0.1 mg/L within different tanks. The highest levels of ammonia were mostly found within the experimental treatments. This is attributed to the decomposing feed, high fat content of the larvae, waste produced by the fish, pH, and temperature. The greater the pH and temperature, the greater the ammonia levels. Once levels are outside of the recommended range it can become toxic to tilapia (Abd El-Hack et al., 2022). As for why there were no high mortality rates, tilapia is known to be a hardy fish with the ability to survive inadequate conditions. However, to avoid such circumstances, a water quality test was done twice a week to determine if the water needed to be changed.

In various cases, nitrate levels exceeded the recommended levels of 1.5 - 2.0 mg/L in different tanks which can become toxic to fish (Shokr, 2015). The highest nitrate levels were found in the experimental diet tanks due to decomposing feed, high fat content of the larvae, and tilapia excreta. Similar results were also recorded for nitrite levels with the highest level being at 5 mg/L or more. According to (Paul et al., 2020), the level of nitrate in the water is also influenced by nitrifying bacteria. Bacterial activity is controlled by the temperature and pH levels. The higher they are, the greater the activity of the bacteria, therefore resulting in greater nitrate levels. These factors were also found to affect nitrite levels as well (Ciji y Akhtar, 2020). Routine water changes minimize mortality.

Most tanks remained within the mentioned limits. Although tilapia can tolerate a diverse range of pH levels, once it is outside of the recommended range of 6.5 - 9 it can reduce the fish's performance (Abd El-Hack et al., 2022).

Conclusions

It was determined that black soldier fly larvae (*Hermetia illucens*) can substitute up to 75% of the protein source in conventional feed for the diets of Nile tilapia fry (*Oreochromis niloticus*) without compromising the growth parameters. This finding applies specifically to the meat meal used during the study.

The use of black soldier fly larvae meal was found to outperform meat meal, resulting in the highest daily weight gain and final mean weight. However, considering that there were no differences in the feed conversion ratio between the experimental and control diets, the recommended inclusion level of black soldier fly meal should be based on other factors, such as its availability, cost, and environmental impact of its production.

Recommendations

Defat the black soldier fly larvae to increase the protein content, improve the texture of the feed, and palatability using an oil extractor machine.

Complete an economic analysis of the actual costs of black soldier fly larvae as an alternative protein source to determine its feasibility within the aquaculture unit.

Complete an environmental analysis to determine whether it is environmentally sustainable to use black soldier fly larvae as an alternative feed in comparison to conventional feeds. This would involve evaluating its carbon footprint and use of natural resources during production.

Reevaluate the study using fish meal as the protein source within the control diet to compare its performance to black soldier fly larvae meal on Nile tilapia.

Conduct a new study using other species of insects with a high protein content to determine their performance on tilapia fry, environmental sustainability, and economic feasibility.

References

- Abd El-Hack, M. E., El-Saadony, M. T., Nader, M. M., Salem, H. M., El-Tahan, A. M., Soliman, S. M. & Khafaga, A. F. (2022). Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). *International Journal of Biometeorology*, 66(11), 2183–2194. <https://doi.org/10.1007/s00484-022-02347-6>
- Baiano, A. (2020). Edible insects: An overview on nutritional characteristics, safety, farming, production technologies, regulatory framework, and socio-economic and ethical implications. *Trends in Food Science & Technology*, 100, 35–50. <https://doi.org/10.1016/j.tifs.2020.03.040>
- Bandara, T. (2018). Alternative feed ingredients in aquaculture: Opportunities and challenges. *Journal of Entomology and Zoology Studies*, 6(2), 3087–3094. <https://www.entomoljournal.com/archives/2018/vol6issue2/PartAJ/6-1-130-287.pdf>
- Belghit, I., Liland, N. S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Krogdahl, Å. & Lock, E.-J. (2019). Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture*, 503, 609–619. <https://doi.org/10.1016/j.aquaculture.2018.12.032>
- Caimi, C., Renna, M., Lussiana, C., Bonaldo, A., Gariglio, M., Meneguz, M., Dabbou, S., Schiavone, A., Gai, F., Elia, A. C., Prearo, M. & Gasco, L. (2020). First insights on black soldier fly (*Hermetia illucens* L.) larvae meal dietary administration in Siberian sturgeon (*Acipenser baerii* Brandt) juveniles. *Aquaculture*, 515, 734539. <https://doi.org/10.1016/j.aquaculture.2019.734539>
- Ciji, A. & Akhtar, M. S. (2020). Nitrite implications and its management strategies in aquaculture: A review. *Reviews in Aquaculture*, 12(2), 878–908. <https://doi.org/10.1111/raq.12354>
- Craig, S. & Helfrich, L. (2017). *Understanding fish nutrition, feeds, and feeding*. Virginia Cooperative Extension. <https://fisheries.tamu.edu/files/2019/01/FST-269.pdf>
- Geehan, J., Zhou, X., Gee, J., Ye, Y., Vannuccini, S., Metzner, R., Zelasney, J., Taconet, M., Mair, G., Hidas, E., Ryder, J., Shen, N., Metzner, R., Castro de Souza, M., van Anrooy, R., Dabbadie, L., Lem, A. & Camilleri, M. (2020). *The state of world fisheries and aquaculture: Sustainability in action. The state of world fisheries and aquaculture: Vol. 2020*. FAO. <https://www.fao.org/3/ca9229en/ca9229en.pdf> <https://doi.org/10.4060/ca9229en>
- Hernández, C. (2021). *Series de precipitación y temperatura en Zamorano: tendencias, proyección y relación con el cambio climático* [Special graduation project]. Escuela Agrícola Panamericana, Zamorano, Honduras. <https://bdigital.zamorano.edu/server/api/core/bitstreams/c833d7a4-b8b3-4585-9b6b-e137878b29d7/content>
- Hodar, A. A., Vasa, R. J., Mahavadiya, D. R. & Joshi, N. H. (2019). Fish meal and fish oil replacement for aquaculture formulation by using alternative sources: A review. *Journal of Experimental Zoology India*, 23(1), 13–21.
- Jonas-Levi, A. & Martinez, J.-J. I. (2017). The high level of protein content reported in insects for food and feed is overestimated. *Journal of Food Composition and Analysis*, 62, 184–188. <https://doi.org/10.1016/j.jfca.2017.06.004>
- Laboratorio de Análisis de Alimentos Zamorano. (2022). *Chemical Proximal Analysis of Hermetia illucens*.

- Limbu, S. M., Shoko, A. P., Ulotu, E. E., Luvanga, S. A., Munyi, F. M., John, J. O. & Opiyo, M. A. (2022). Black soldier fly (*Hermetia illucens*, L.) larvae meal improves growth performance, feed efficiency and economic returns of Nile tilapia (*Oreochromis niloticus*, L.) fry. *Aquaculture, Fish and Fisheries*, 2(3), 167–178. <https://doi.org/10.1002/aff2.48>
- Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., Purba, R. A. P. & Paengkoum, P. (2022). Nutritional composition of black soldier fly larvae (*Hermetia illucens* L.) and its potential uses as alternative protein sources in animal diets: A Review. *Insects*, 13(9), 1–17. <https://doi.org/10.3390/insects13090831>
- Mengistu, S. B., Mulder, H. A., Benzie, J. A. H. & Komen, H. (2020). A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture*, 12(2), 524–541. <https://doi.org/10.1111/raq.12331>
- Munguti, J. M., Nairuti, R., Iteba, J. O., Obiero, K. O., Kyule, D., Opiyo, M. A., Abwao, J., Kirimi, J. G., Outa, N., Muthoka, M., Githukia, C. M. & Ogello, E. O. (2022). Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) culture in Kenya: Emerging production technologies and socio-economic impacts on local livelihoods. *Aquaculture, Fish and Fisheries*, 2(4), 265–276. <https://doi.org/10.1002/aff2.58>
- Nagappan, S., Das, P., AbdulQuadir, M., Thaher, M., Khan, S., Mahata, C., Al-Jabri, H., Vatland, A. K. & Kumar, G. (2021). Potential of microalgae as a sustainable feed ingredient for aquaculture. *Journal of Biotechnology*, 341, 1–20. <https://doi.org/10.1016/j.jbiotec.2021.09.003>
- Paul, I., Panigrahi, A. K. & Datta, S. (2020). Influence of nitrogen cycle bacteria on nitrogen mineralisation, water quality and productivity of freshwater fish pond: A Review. *Asian Fisheries Science*, 33(2). <https://doi.org/10.33997/j.afs.2020.33.2.006>
- Perez-Velazquez, M., Millanes-Mora, M. A. & González-Félix, M. L. (2024). Assessment of hydrolysis of partially defatted black soldier fly larvae meal in diets for Nile tilapia *Oreochromis niloticus*. *Animal Feed Science and Technology*, 307, 115831. <https://doi.org/10.1016/j.anifeedsci.2023.115831>
- Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M. T., Biasato, I., Biasibetti, E., Marco, M. de, Brugiapaglia, A., Zoccarato, I. & Gasco, L. (2017). Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *Journal of Animal Science and Biotechnology*, 8, 57. <https://doi.org/10.1186/s40104-017-0191-3>
- Shokr, E.-S. A. (2015). Effect of ammonia stress on blood constituents in Nile tilapia. *Egyptian Academic Journal of Biological Sciences, B. Zoology*, 7(1), 37–44. 10.21608/eajbsz.2015.13476
- Sumaila, U. R. & Tai, T. C. (2020). End overfishing and increase the resilience of the ocean to climate change. *Frontiers in Marine Science*, 7, Artículo 523, 1–8. <https://doi.org/10.3389/fmars.2020.00523>
- Tippayadara, N., Dawood, M. A. O., Krutmuang, P., Hoseinifar, S. H., van Doan, H. & Paolucci, M. (2021). Replacement of fish meal by black soldier fly (*Hermetia illucens*) larvae meal: Effects on growth, haematology, and skin mucus immunity of Nile tilapia, *Oreochromis niloticus*. *Animals : An Open Access Journal from MDPI*, 11(1). <https://doi.org/10.3390/ani11010193>

- Turchini, G. M., Ng, W.-K. & Tocher, D. R. (2010). *Fish oil replacement and alternative lipid sources in aquaculture feeds* - Google Books (1^a ed.). <https://doi.org/10.1201/9781439808634>
- United Nations. (n.d.). *Population / United Nations*. <https://www.un.org/en/global-issues/population>
- van Dijk, M., Morley, T., Rau, M. L. & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010-2050. *Nature Food*, 2(7), 494–501. <https://doi.org/10.1038/s43016-021-00322-9>
- Yanbo, W., Wenju, Z., Weifen, L. & Zirong, X. (2006). Acute toxicity of nitrite on tilapia (*Oreochromis niloticus*) at different external chloride concentrations. *Fish Physiology and Biochemistry*, 32(1), 49–54. <https://doi.org/10.1007/s10695-005-5744-2>