Article DOI: https://doi.org/10.37284/eajab.7.1.1716



Original Article

# Integrating Black Soldier Fly (Hermetia illucens) Larvae Meal in Aquaponics System for Lettuce (Lactuca sativa) Production

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Article DOI: https://doi.org/10.37284/eajab.7.1.1716

Date Published: ABSTRACT

24 January 2024

Keywords:

Aquaponics, Hydroponics, Aquaculture, Lettuce, Black Soldier Fly, Fishmeal. The adoption of aquaponics systems faces challenges due to the high cost of fish feed, lack of sustainability, and reliance on fishmeal as a primary input. One potential solution to replace fishmeal is the use of black soldier fly larvae (BSFL) in aquaponics. The study aimed to explore the effect of substituting fish meals with BSFL meals on the growth performance of lettuce (Lactuca sativa) in aquaponics. The study employed the Randomized Complete Block Design (RCBD). A total of 135 catfish fingerlings were divided into three groups and stocked in nine water barrels of 100 litres each (filled with 60 litres of water). The fingerlings were fed twice a day for a duration of 56 days after a two-week period of acclimatisation using commercial feed. Water was cycled through the hydroponic media grow bed using an automatic timer, with a 15-minute cycle every hour throughout the day and night. Fish meal was replaced with BSFL meal at three different proportions: 0% (BSFL0), 30% (BSFL30), and 50% (BSFL50). The data collected was analysed using One-Way Analysis of Variance (ANOVA) and Kruskal-Wallis tests, followed by Tukey's ad-hoc and Dunn's tests, respectively, utilising JMP 16 software. The results indicated that the fresh leaf weight was significantly influenced by the diet types (p<0.05). However, no significant effect was observed on root weight, total dry weight, number of leaves, or root length (p>0.05). This study suggests that black soldier fly larvae meal can successfully replace up to 50% of fishmeal in aquaponics without compromising lettuce growth performance. Therefore, promoting the use of black soldier fly larvae meal can contribute to the sustainability of lettuce production in aquaponics.

#### APA CITATION

Nguka, S. O., Mweresa, C. K. & Muriithi, A. N. (2024). Integrating Black Soldier Fly (Hermetia illucens) Larvae Meal in Aquaponics System for Lettuce (Lactuca sativa) Production. *East African Journal of Agriculture and Biotechnology*, 7(1), 62-71. https://doi.org/10.37284/eajab.7.1.1716

#### CHICAGO CITATION

Nguka, Samuel Ochieng, Collins Kalwale Mweresa and Alice Nakhumicha Muriithi. 2024. "Integrating Black Soldier Fly (Hermetia illucens) Larvae Meal in Aquaponics System for Lettuce (Lactuca sativa) Production". *East African Journal of Agriculture and Biotechnology* 7 (1), 62-71. https://doi.org/10.37284/eajab.7.1.1716

#### HARVARD CITATION

Nguka, S. O., Mweresa, C. K. & Muriithi, A. N. (2024) "Integrating Black Soldier Fly (Hermetia illucens) Larvae Meal in Aquaponics System for Lettuce (Lactuca sativa) Production", *East African Journal of Agriculture and Biotechnology*, 7(1), pp. 62-71. doi: 10.37284/eajab.7.1.1716.

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Article DOI: https://doi.org/10.37284/eajab.7.1.1716

#### **IEEE CITATION**

S. O. Nguka, C. K. Mweresa & A. N. Muriithi "Integrating Black Soldier Fly (Hermetia illucens) Larvae Meal in Aquaponics System for Lettuce (Lactuca sativa) Production", *EAJAB*, vol. 7, no. 1, pp. 62-71, Jan. 2024.

#### MLA CITATION

Nguka, Samuel Ochieng, Collins Kalwale Mweresa & Alice Nakhumicha Muriithi. "Integrating Black Soldier Fly (Hermetia illucens) Larvae Meal in Aquaponics System for Lettuce (Lactuca sativa) Production". *East African Journal of Agriculture and Biotechnology*, Vol. 7, no. 1, Jan. 2024, pp. 62-71, doi:10.37284/eajab.6.1.1716.

## INTRODUCTION

The projected global population of around 9.7 billion by 2050, along with the impact of climate change, highlights the need for adopting sustainable and alternative food production systems (FAO et al. 2018). Three such systems; aquaculture, hydroponics, and aquaponics, have the potential to increase food production efficiently while conserving land, water, and other natural resources (Kyaw & Ng, 2017). Aquaculture involves raising aquatic animals or cultivating aquatic plants for food and plays a vital role in providing protein through fish production and creating employment opportunities (Mapfumo, 2022). However, to ensure sustainability in aquaculture, it is essential to address the treatment of nutrient-rich water and reduce reliance on expensive fish meal (Somerville et al., 2014). The quality, availability, and cost of fish feeds affect aquaculture development in Kenya (Munguti et al., 2021).

Hydroponics is a method of growing plants without soil using a nutrient-supplied growing medium. The hydroponic systems still require the formulation of costly nutrients that necessitate technical skills and knowledge (Knaus & Palm, 2017; Koop, 2016). Aquaponics combines aquaculture and hydroponics by integrating fish production with soilless plant cultivation in a system that involves water circulation (Knaus & Palm, 2017; Koop, 2016). It has gained attention for its potential to address food security, water conservation, and efficient use of natural resources. The various aspects of aquaponics systems that have been studied include system design, nutrient cycling, water quality, and energy usage, among others (Badiola et al., 2018; Buzby & Lin, 2014). These studies aim to optimise system performance for nutrient cycling, plant and fish growth, as well as environmental and

economic sustainability (Love et al., 2015). Previous research in aquaponics has assessed the growth rates of different fish species, plant yield, and nutritional content. It has also explored factors influencing plant growth performance, light intensity and nutrient such as supplementation. Additionally, research has focused on nutrient dynamics within aquaponics systems, including nutrient concentrations, plant uptake rates, and nutrient availability for fish (Rakocy et al., 2016). These studies aimed to determine the ideal nutrient ratios for maximising plant growth and fish health.

Furthermore, fish health, growth, welfare, effects of stocking density, and feed composition on fish performance have also been investigated (Somerville et al., 2014; Yildiz et al., 2017). The influence of nitrifying bacteria on nutrient cycling and overall system health in aquaponics systems has been examined as well (Schmautz et al., 2017). Recent studies have examined the integration of aquaponic systems into urban agriculture, with a focus on supplying fresh produce and fish in densely populated areas. These studies have specifically looked at how aquaponics can be adapted to rooftop gardens, vertical farming, and other urban spaces. The applicability of aquaponics in small-scale and urban environments has been explored, space limitations, considering community involvement, and food security. However, little research has been conducted on alternative ingredients that can replace fish meal. In aquaponics, plants receive nutrients from fish faeces and leftover feed.

Achieving balanced and sustainable plant growth requires careful consideration of the feed rate ratio (Somerville et al., 2014). Black soldier fly larvae (BSFL) meal has gained attention as a potential protein source in various industries, including Article DOI: https://doi.org/10.37284/eajab.7.1.1716

aquaponics. Incorporating BSFL meal into aquaponic systems has the potential to improve nutrient cycling, enhance system sustainability, and provide a valuable feed source for fish and plants. However, more research and data are needed specifically on the use of black soldier fly larvae meal in aquaponic systems. This would help determine the optimal inclusion rate and understand the long-term effects on the entire aquaponic ecosystem. Different combinations of fish species and vegetables have shown varying results, suggesting that careful consideration should be given to the choice of fish and crops used in aquaponics (Knaus & Palm, 2017). Several crops, such as cauliflower, eggplant, peppers, beans, peas, cabbage, broccoli, Swiss chard, parsley, and lettuce, have the potential to be cultivated in aquaponic systems (Somerville et al., 2014).

In aquaponics, the well-being of both fish and plants is interconnected. Therefore, the use of a new feed source, such as black soldier fly larvae meal, may affect the nutrient balance in the system, potentially influencing plant growth and health. It is essential to investigate the effects of using BSFL meals in aquaponics. As a result, this study evaluates the effects of replacing fish meals with BSFL meals on the performance of lettuce (Lactuca sativa) in an aquaponics system.

#### MATERIALS AND METHODS

#### **Study Area**

This study was carried out at Miramar International College located in Muthiga, Munyua Road, off Waiyaki Way, Kikuyu Sub-County in Kiambu County. The college is located at Latitude 1°15'18.1" S, and Longitude 36° 41'10.0" E. The average annual temperature and rainfall for Kiambu County are 15-23°C and 600-1300mm, respectively (MoALFC, 2021).

#### System Design

Low-cost models of aquaponics systems were constructed using the same design, which consisted of two parts: a fish tank and hydroponic media grow beds. The setup was placed inside a greenhouse for security purposes and to protect against external factors such as rain. The design included a recirculatory water system, with nine water barrels (Roquette India Pvt Ltd) procured from the Kariakor market in Nairobi, each connected grow to а bed (barrel). The fish tanks were created using nine barrels (Roquette India Pvt Ltd), each with a capacity of 100 litres, and were placed on building blocks at a lower height than the connected grow beds. The growing beds had a capacity of 125 litres each and were filled with pumice. The building blocks helped to reduce the height difference between the fish tanks and the grow beds to meet the maximum height requirement for the pump. For pumping water to the hydroponic media beds, a JY-815 pump (AC 220-240, 50Hz, 20W, H.1.2M, F MAX 1200L/H) and a SHANDA SDP-800 (Shanda®) pump (AC 220-240, 2W, H.MAX 1.6, F.MAX 1.6M) were used. The SHANDA SDP-800 pump replaced the JY-815 pump, which broke down during the course of the experiment. To ensure that there was sufficient oxygen available for the fish in the tanks, air pumps (RSElectical®) (Rs-1000: 220-240V, 9 litres/minute output, 8W) and air stones (Aquaneat®) were used to supplement dissolved oxygen.

#### Initial Cycling for a Balanced System

Water from an existing fishpond, which already contained levels of ammonia, nitrites, nitrates, and bacteria, was added (2 litres per tank) to the system to speed up maturing. The water quality parameters were taken daily and recorded to monitor the concentration of ammonia, nitrites, and nitrates, as well as levels of temperature, pH, and EC. To test the system balance, a few catfish fingerlings (2 per tank) were added to the tanks after seven days when the levels of ammonia started to decline, and nitrites and nitrate levels increased. During this initial cycling period, the fish were fed twice daily (2% of their body weight). A few lettuce seedlings (2 seedlings per grow bed) were also introduced (planted) at week 2, during this time, as nitrate levels began to rise. For the cycling, which would later be adopted for the entire experiment, the water pumps were

Article DOI : https://doi.org/10.37284/eajab.7.1.1716

connected to an automatic timer that allowed the pumping of water from the fish tank into the grow bed for 15 minutes every 45 minutes for 24 hours. The water containing metabolic wastes was thus pumped from the fish tank into the lettuce grow beds, and the purified water drained back to the fish tank. The system was considered fully cycled (balanced) when ammonia and nitrite levels were reduced to <1 mg/L and nitrate levels increased to more than 100 mg/L.

#### **Experimental Design**

Randomised Complete Block Design (RCBD) was used in this trial. African catfish fingerlings weighing an average of 25 g each were randomly distributed to 9 fish tanks (100-litre barrels) at a stocking density of 15 fish per tank. The setup, consisting of nine fish tanks, each connected to a grow bed, was divided into three treatments, each performed in triplicate. The three treatments were determined by the type of feed used and labelled as follows: BSFL0 (100% fish meal, 0% BSFL), BSFL30 (70% fish meal, 30% BSFL), and BSFL50 (50% fish meal, 50% BSFL). Based on these treatments, three different diet types containing black soldier fly larvae (BSFL) were formulated to replace 0%, 30%, and 50% of the fishmeal. The diet containing fish meal (treatment BSFL0) was used as the control for the experiment.

# Monitoring and Measuring Water Quality Parameters

During the initial system cycling period, pH and temperature measurements were taken daily. In addition, nitrogen levels were tested daily using water testing kits to monitor the levels of ammonia, nitrite, and nitrate. Temperature, pH, and Electrical Conductivity were measured using the Hanna Combo pH meter (Combo®) (Amiran Kenya). Ammonia concentration was measured using Salifert ammonia test kits (Salifert®) (Samaki Express, Nairobi, Kenya). Nitrite content was measured using the Easy Strips<sup>TM</sup> tetra test kit (Samaki Express, Nairobi, Kenya). The tests were done weekly when the system showed stability.

# Feeds Used, Feeding Frequency, Feeding Level and Weighing

The BSFL Meal (sun-dried) was purchased from a local farmer in Kajiado County. Other ingredients used to ensure a complete diet with all the required nutrients were prepared, including wheat pollard, trace minerals, vitamins, and binders. Fish feed containing fish meal as the main protein source was used as a control diet in the experiment. For acclimatisation before the commencement of the study, the fingerlings were fed commercial feed obtained from Sigma feeds in Nairobi, Kenya, for two weeks.

Ingredients	Diet			
	<b>BSFL0 (%)</b>	<b>BSFL30</b> (%)	<b>BSFL50</b> (%)	
Fish meal	70.5	43.1	60.4	
BSFL	0.0	43.1	25.9	
Wheat pollard	29.2	13.5	13.5	
Binder	0.1	0.1	0.1	
Vitamin premix	0.1	0.1	0.1	
Trace mineral	0.1	0.1	0.1	
Total	100.0	100.0	100.0	

Table 1: Percentage composition of ingredients in the formulated feeds

Experimental diets included different levels of Black soldier fly larvae (BSFL) meal replacement at 0% BSFL meal + 100% fishmeal (Control diet), 30% BSFL meal + 70% fishmeal, 50% BSFL meal + 50% fishmeal. The fish were fed twice daily between 7:00-9:00 am and 18:00-19:30 at the rate of 2% of their body weight.

Article DOI: https://doi.org/10.37284/eajab.7.1.1716

# Observing Lettuce Plants and Measuring Weights

Root length =  $\frac{Sum of root lengths measured}{Number of lettuce plants measured}$ 

Part of the activities done during the experiment included monitoring and observing the growth of lettuce grown in this system. The infected lettuce plants were removed from the system when identified. Growth-related parameters such as average leaf Fresh weight, Leaf Dry Weight, root weight, and root length were measured per plant per treatment, while the average number of leaves/plant/treatments was also determined at harvest after 56 days. Lettuce plants were weighed at harvest (after 56 days) using a digital weighing machine to determine the weight gained over the experimental period. At the end of the experiment, the lettuce plants were carefully removed from the grow bed, roots and leaves were separated, washed and blotted using a soft paper to remove moisture on the surface of the leaves, then dried in an oven overnight at 40 °C.

# **Calculations**

The data that was collected from the experiment were used to calculate the following performance indicators critical in determining the growth of lettuce.

Average Fresh weight (leaf, root) = <u>Total weight of leaves,root</u> <u>No of leaves,roots weighed</u>

Dry weight (leaf, root) = Total dry weight of leaves,roots Number of leaves,roots weighed

# **Statistical Analysis**

Data was collected and recorded in Excel. A normality check was done, and then the data was subjected to One Way Analysis of Variance (ANOVA) and Kruskal Wallis test to determine significant differences between treatments. The difference in the performance of lettuce (leaf dry weight and root weight) grown under the three treatments was determined using ANOVA. Leaf wet weight, total weight and root length were analysed through the Kruskal Wallis test. The data was then subjected to the Dunn test for pairwise comparison of fresh leaf weight from fish meal versus BSFL30 and BSFL50. All analyses were conducted in JMP Software version 16 at a 95% confidence level.

# RESULTS

Diet types significantly affected the growth performance of lettuce in the aquaponics system (p<0.05) (*Table 2*). The fresh leaf weight of lettuce was significantly influenced by the diets (p<0.05). While the fresh leaf weight of lettuce grown under BSFL0 was not different when compared to BSFL50 (p>0.05), there was a significant difference (p<0.05) in the fresh leaf weight of lettuce grown under BSFL30 when compared to both BSFL0 and BSFL50 (*Table 2*).









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## Fresh Root Wet Weight

The diet types used did not affect the fresh root weight of lettuce in the system (p>0.05). The fresh root weight of lettuce was not significantly different (p>0.05) when BSFL0 was compared to BSFL30 and BSFL50. Similarly, there was no significant difference (p>0.05) between the fresh root weight of lettuce grown under BSFL30 and BSFL50 (*Table 2*).

# **Total Plant Fresh Weight**

The total fresh weight of lettuce was significantly influenced by diet types (p<0.05). The total fresh weight of lettuce grown under BSFL30 was comparatively lower than BSFL0 and BSFL50. However, there was no difference between the total fresh weight of lettuce grown under BSFL0 and BSFL50 (p>0.05) (*Table 2*).

# Plant Leaf and Root Dry Weights

Furthermore, diet types had no effect on leaf dry weight (p>0.05). The highest root dry weight was recorded in a system where BSFL0 was used, followed by the BSFL50 diet, but the difference was not significant. The least root dry weight was observed in the hydroponic system connected to fish tanks containing the BSFL30 diet. However, the differences were statistically similar (p>0.05). The effects of the different diet types were compared among the treatments.

# **Total Plant Dry Weight**

The total dry weight of lettuce grown under the BSFL50 diet was not significantly different (p>0.05) when compared between BSFL0 and BFSL30. However, there was a significant difference between the weights recorded between the BSFL0 diet and BSFL30 (p<0.05) (*Table 2*).

# **Root Length of Lettuce**

The diets used to feed catfish did not significantly affect the root length of lettuce (p>0.05). There was no significant difference (p>0.05) in the root length of lettuce when BSFL0 was used compared to BSFL30 and BSFL50. Similarly, the root length observed when BSFL30 was used was not different when compared to BSFL50 (p>0.05) (*Table 2*).

# Number of Leaves

The diets did not significantly affect the number of lettuce leaves grown (p>0.05). Thus, the number of leaves in beds supplied with BSFL0 was not different when compared to both BSFL30 and BSFL50. Also, there was no significant difference between the number of lettuce leaves provided with water containing the BSFL30 diet compared to BSFL50 (*Table 2*).

Growth Parameter		Treatm	Treatment type	
	BSFL0	BSFL30	BSFL50	
Total fresh Weight	413.33±2.40 <sup>a</sup>	320±9.71 <sup>b</sup>	386.67±5.93 <sup>a</sup>	0.001
Total Dry Weight	$20.42 \pm 0.60^{a}$	$17.70 \pm 0.18^{b}$	$19.07 \pm 0.84^{ab}$	0.05
Fresh leaf Weight	378.33±3.38 <sup>a</sup>	$290.67 \pm 9.87^{b}$	355.33±7.22 <sup>a</sup>	0.001
Fresh root Weight	35.00±0.58ª	29.33±1.20 <sup>a</sup>	$31.33 \pm 1.86^{a}$	0.06
Leaf Dry Weight	$18.45 \pm 0.48^{a}$	$16.20\pm0.18^{a}$	17.43±0.75 <sup>a</sup>	0.06
Root Dry Weight	$1.97{\pm}0.18^{a}$	1.5±0.1 <sup>a</sup>	1.63±0.09 <sup>a</sup>	0.09
Number of leaves	$20.7 \pm 1.2^{a}$	$18.0\pm0.6^{a}$	20.3±0.9 <sup>a</sup>	0.16
Root Length (cm)	$8.1 \pm 0.81^{a}$	6.73±0.37 <sup>a</sup>	$8.83 \pm 0.09^{a}$	0.07

 Table 2: Growth parameters of lettuce raised in an aquaponic system where catfish fingerlings

 were fed separately on either of the three diet types over a period of eight weeks

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#### DISCUSSION

#### Lettuce Fresh and Dry Leaf Weight

The fresh leaf weight of lettuce grown under BSFL0 and BSFL50 were similar (Table 2). However, BSFL30 significantly influenced the fresh leaf weight of lettuce (Table 2). The significant difference in lettuce leaf weight grown under BSFL30 can be attributed to the lower concentration of nitrogen (Maucieri et al., 2018). The lower concentration of nutrients in the system can possibly be explained by the yellowing and slower growth of lettuce grown under BSFL30. However, the different diet types had no effect on leaf dry weight. Dry weight is considered to be a more reliable measure of plants' growth performance since plants have a high composition of water, and the level of water depends on the amount of water in its environment. This explains the significant effect obtained in fresh leaf weight of lettuce when subjected to BSFL0, BSFL30, and BSFL50, with dry weights of lettuce being similar for all the treatments. However, the fresh weight of lettuce is important to farmers since it is the one considered in determining the profitability of lettuce production.

# Fresh and Dry Root Weight.

The results showed that the highest dry root weight was in the treatment BSFL0 diet system, followed by the BSFL50 diet (*Table 2*). Further analysis revealed that the root dry weight of lettuce was not significantly influenced by the treatments (*Table 2*). These results were comparable to those reported by Al Tawaha et al. (2021). Furthermore, the root biomass was within the range of 22g-35g/plant obtained from previous experiments using nutrient supplementation (Nozzi et al., 2018).

# **Total Wet and Dry Weight**

The total fresh weight obtained from this study was also higher than those reported previously (Al Tawaha et al., 2021; Johnson et al., 2017; Jordan et al., 2017; Kovácsné Madar et al., 2019; Luo et al., 2021; Yeşiltaş et al., 2021). The higher performance can be attributed to sufficient nutrient supply in the system compared to the previous studies. The differences in lettuce production can be attributed to different varieties of lettuce, environmental conditions, nutrient concentration, water temperatures, and solar radiation (Bordignon et al., 2022). The variety of lettuce grown in this study was romaine lettuce, unlike other studies which used butterhead lettuce; hence the varieties may have contributed to the observed difference Al Tawaha et al. (2021). The variation in the performance in this study could also be attributed to the growing media used in the system (Yeşiltaş et al. 2021). This study used a media grow bed system involving pumice. Pumice contains carbonates that occasionally raise the pH of the culture water. Two tablespoons of fresh lemon were used to lower the pH at stocking. However, the lowered pH levels were not maintained as they increased above 7.5 within three hours. The resultant high pH levels could have limited nutrient absorption, hence lower growth performance compared to studies. Studies with other nutrient supplementation performed better than this study as it depended fully on nutrients resulting from fish wastes and feed residues, hence lower nutrient concentration compared with previous studies (Luo et al., 2021). The current study was solely dependent on fish feed to supply nutrients to both the aquaculture and hydroponic components of the system. Low yields of lettuce in aquaponics are associated with low levels of phosphorus, potassium, iron, and manganese (Al Tawaha et al., 2021; Rakocy et al., 2016). The total dry weight of lettuce grown under the BSFL50 diet was not significantly different when compared between BSFL0 and BFSL30. However, there was a significant difference between the weights recorded between the BSFL0 diet and BSFL30 (Table 2).

## **Root Length**

Over the 56-day study period, the observed lettuce root length showed that the ones under BSFL50 treatment were longer followed by BSFL0 and then BSFL30 (*Table 2*). However, there was no significant difference in lettuce root length

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between the treatments. The results of root length in this study were comparable to those reported by Al Tawaha et al. (2021). The final root length obtained in the current study was lower than those reported by Yeşiltaş et al. (2021). The lower root length compared to previous studies may imply that nutrients were readily available and there were no deficiencies in micronutrients and phosphorus in the system, which determines the root-to-shoot ratio (Delaide et al., 2016; Nozzi et al., 2018).

# **Number of Leaves**

The initial counts showed that treatment BSFL0 and BSFL50 had an almost similar number of leaves, followed by treatment BSFL30. However, the analysis revealed that the treatments did not significantly affect the number of lettuce leaves grown (p>0.05) (*Table 2*). Thus, the number of leaves in beds supplied with BSFL0 was not different when compared to both BSFL30 and BSFL50. These results are comparable to those reported by (Al Tawaha et al., 2021). From observations, the lettuce plants grown under treatment BSFL30 diets showed observable stunted growth and yellowing. This stunted growth could possibly be attributed to insufficient levels of macronutrients such as phosphorus, potassium, magnesium, and calcium (Al Tawaha et al., 2021; Maucieri et al., 2018). Yellowing was observed in the lettuce grown under BSFL30 during the experimental period compared to BSFL0 and BSFL50. This can be attributed to inadequate levels of iron and nitrogen in the solution compared to the levels produced when BSFL0 and BSFL50 were used (Al Tawaha et al., 2021; Maucieri et al., 2018). The current study focused on the concentration of ammonia, nitrites, and nitrates, which are products of the nitrification process. The results have indicated that replacing 30% and 50% fish meal with black soldier fly larvae meal does not statistically influence the nitrification process in aquaponics systems.

# CONCLUSION

Replacing 30% of fish meal with black soldier fly larvae in aquaponics significantly reduced the

fresh leaf weight of lettuce, but other growth parameters of lettuce were not influenced. Thus, a 50% replacement of fish meal in content with BSFL can optimally support lettuce production in aquaponics systems.

# ACKNOWLEDGMENT

This research was funded by Jaramogi Oginga Odinga University of Science and Technology through the African Centre of Excellence in sustainable use of insects as feed and food (INSEFOODS).

## **Ethical Approval**

This study was approved by the Ethics Review Committee (ERC) of Jaramogi Oginga Odinga University of Science and Agriculture and the National Commission for Science Technology and Innovation (NACOSTI)

# **Conflict Of Interest**

The author declares that there is no conflict of interest.

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