**EFFECT OF BLACK SOLDIER FLY (*Hermatia illucens*) LARVAE MEAL WITH CITRUS PEELS ON THE GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF AFRICAN CATFISH.**

**ABSTRACT**

The effects of completely substituting Black Soldier Fly for fishmeal and adding orange, tangerine, or tangelo peel as phyto-additives on the development, nutrient consumption, and hematology of juvenile *Clarias gariepinus* were investigated in a 70-day experiment. Seven diets containing 40% crude protein using fishmeal (25% contributed protein) in the control diet while the fishmeal was replaced equi-protein in the six other diets from Black Soldier Fly (BSF) larvae. Three of the six diets were supplemented with either 1 or 2% of either orange, tangerine, or tangelo peel (ORG1, TGR1 TGL1, ORG2, TGR2, and TGL2, respectively).  315 juveniles were distributed into 21 plastic bowls each containing 15 (8.48 to 8.57g/fish) of African catfish (*Clarias gariepinus*). Fish-fed TGR2 produced the lowest values but the highest FCR, while fish-fed the control diet responded best in the following growth indices: FMW, MWG, PWG, SGR, FCR, FER, PER, and Survival (97.77%). The FMW, MWG, PWG, and SGR values of fish fed the control and the other diets differed significantly (p < 0.05). The physicochemical parameters for *Clarias gariepinus* were all within the optimal range. Although rich in protein, BSF may not be an ideal standalone protein source. However, citrus peels, especially orange peels, when incorporated into the diet, could serve as an effective additive for promoting sustainable aquaculture feeds, provided they are paired with more complete protein sources to ensure adequate nutritional intake.

Keywords: African Catfish, Black Soldier Fly Larvae, Citrus peels, Growth and nutrient Utilization

**ABBREVATIONS**

ORG 1- Black soldier fly larvae meal with 1% orange peel

TGR 1- Black soldier fly larvae meal with 1% tangerine peel

TGL 1 - Black soldier fly larvae meal with 1% tangelo peel

ORG 2- Black soldier fly larvae meal and 2% orange peel

TGR 2 - Black soldier fly larvae meal and 2% tangerine peel

TGL 2 - Black soldier fly larvae meal and 2% tangelo peel

FI- Feed Intake,

FCR -Feed Conversion Ratio

FER - Feed Efficiency Ratio

PER - Protein Efficiency Ratio

S- Survival Percentage

**1.0 INTRODUCTION**

The world aquaculture output has grown tremendously and currently contributes over half (about 53%) of the total human consumed food fish (FAO, 2020). The domestic supply of fish was estimated at 2.13 million metric tons (MMT) in 2018, compared to the 3.61 MMT fish demand for the same year. This results in a deficit of more than 1.5 MMT, which could only be filled by aquaculture. Aquaculture's contribution to global food fish exceeds 50%, surpassing that of capture fisheries, whose production has generally declined over the years (Verdegem *et al*., 2023).

*Clarias gariepinus* is an important aquaculture fish in Africa and Nigeria, but its culture is being hampered and becoming unattractive due to the high cost and scarcity of feeds (Ed-Idoko *et al*., 2024). However, the increasing demand for fish has led to the overexploitation of fishmeal and fish oil, which are the primary ingredients in fish feeds (Majluf *et al*., 2024). Therefore, the search for alternative sources of protein and energy to replace fishmeal and fish oil in fish feeds has intensified in recent years (Aragão *et al*., 2022).

The black soldier fly, *Hermetia illucens L*., is an insect species used as an unconventional protein source for fish feeding (Lu *et al*., 2022). Its sustainability is related to its capacity to convert organic waste material into biomass containing proteins (40–45%) with high biological value, fat (30–35%) and ash (11–15%) with a high Ca/P ratio (Shah *et al*., 2022). The larvae meal also has a low environmental impact and can be produced from various organic wastes, making it a sustainable and cost-effective alternative protein source for aquafeeds.

Natural compounds from photogenic sources are becoming increasingly popular in aquaculture as growth enhancers and activators of the innate immune system (Ahmadifar *et al.*, 2021). There are numerous bioactive substances in plants that have potential health benefits (Samtiya *et al*., 2021). These bioactive substances include polyphenols, alkaloids, phytosterols, and terpenoids. Some of these bioactive compounds increase the release of digestive enzymes, feed intake, and strengthen immunological responses.

Orange (*Citrus sinensis*) is one of the most popular and widely consumed fruits worldwide, known for its tangy-sweet taste, vibrant color, numerous health benefits, and economic importance (Hazarika, 2025). It belongs to the *Rutaceae* family and is primarily grown in tropical and subtropical regions, with major production areas including Brazil, the United States (particularly Florida and California), Mexico, and India (Mabberley, 2023). The fruit's widespread cultivation and utilization make it a crucial crop in both the global food industry and the agricultural economy (Micha *et al*., 2017). Tangerines belong to the *Rutaceae* family and are a type of mandarin orange (*Citrus reticulata*), which is believed to have originated in Southeast Asia (Palangasinghe *et al*., 2024). It is widely cultivated worldwide in regions with subtropical and tropical climates (Dawson, 2004). The fruit is up to 8cm in diameter with easily separable segments (Boughendjioua and Boughendjioua, 2017). The tangerine (*Citrus reticulata*) is known for its sweet, tangy flavor and easy-to-peel skin. Tangerines are commonly enjoyed fresh but also used in various processed forms, offering significant nutritional benefits, particularly high levels of vitamin C (USDA, 2019).

The tangelo (*Citrus tangelo*) is a hybrid citrus fruit resulting from the cross between a tangerine (*Citrus reticulata*) and a pomelo (*Citrus maxima*) or grapefruit (*Citrus paradisi*) (Galles, 2005). Tangelos are popular for their sweet-tart flavor, easy-to-peel skin, and juicy segments, making them a refreshing choice for a variety of culinary uses (Dawson, 2004; USDA, 2019). The fruit combines the best attributes of its parent species, including the easy-to-segment nature of the tangerine and the larger size and tangy flavor of the pomelo or grapefruit (Dawson, 2004).

Citrus peel is a byproduct of the citrus fruit processing industry and is rich in bioactive compounds such as flavonoids and carotenoids, which have antioxidant and antimicrobial properties (Zulkifli *et al*., 2022). Citrus peels, often discarded in food processing, are rich in a variety of bioactive compounds that have been shown to offer numerous health benefits. These peels contain high levels of essential oils, flavonoids, dietary fiber, and other compounds with potential medicinal and nutritional properties (Cushnie and Lamb, 2005; Slavin, 2013; Li *et al*., 2015).

The study is expected to contribute to the sustainable growth of the aquaculture sector and offer important insights into the potential of black soldier fly larvae meal with citrus peel as substitute protein and energy sources for aquafeeds.

**2.0 MATERIALS AND METHODS**

**2.1       Study Area**

The study was conducted at the Federal University of Technology, Akure, Ondo State, Nigeria, in the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology (Aina *et al*., 2021).

**2.2       Samples Collection**

Black Soldier fly larvae were obtained from His Grace Fisheries (Sumbule *et al*., 2021). The larvae were cleaned, rinsed thoroughly, and oven-dried at 80°C as recommended by Banjo *et al*. (2006). Sweet oranges (*Citrus sinensis*), tangelo (*Citrus × tangelo*), and tangerine (*Citrus reticulata*) fruits were obtained from a fruit seller at the School of Agriculture and Agricultural Technology, Federal University of Technology, Akure. The orange, tangerine, and tangelo peels were spread out and allowed to air dry after the citrus pulps were gently scraped out (Kupagme, 2019). The dried peels were ground into powder using a Binatone electric blender (Model BLG 402) and stored in an airtight container (Owoseni-Fagbenro *et al*., 2024). The ground orange, tangerine, and tangelo peel powders were brought to the Federal University of Technology, Akure's Chemistry Laboratory for mineral analysis. Citrus peel mineral contents were measured according to AOAC (2000) methods.

**2.3       Preparation of Experimental Diets.**

The following ingredients were utilized to create the diets: blood meal, fishmeal, soybean meal, groundnut cake, yellow maize, cassava flour (as a binder), vitamin C, vitamin-mineral premix, orange peel, tangerine peel, tangelo peel, and groundnut oil. Using a combination of these ingredients, seven iso-nitrogenous diets (40% crude protein) were formulated as shown in Table 1. Fishmeal content in the control diet was replaced (100%) with Black Soldier fly larvae meal in all the test diets. Citrus peels were used to replace vitamin-mineral premix at 2% and 1% levels. The experimental diets were: CONTROL (consisting of fishmeal and vitamin-mineral premix), ORG 2 (Black Soldier fly larvae meal and 2% orange peel), TGR 2 (Black Soldier fly larvae meal and 2% tangerine peel), TGL 2 (Black Soldier fly larvae meal and 2% tangelo peel), ORG 1 (Black Soldier fly larvae meal with 1% orange peel), TGR 1 (Black Soldier fly larvae meal with 1% tangerine peel), and TGL 1 (Black Soldier fly larvae meal with 1% tangelo peel).

**2.4       Experimental Setup**

A 2 × 2 factorial experiment in Randomized Complete Block Design (RCBD) was used for the study (Alkutubi *et al*., 2024). Three hundred and fifteen *Clarias gariepinus* juveniles (8.48±0.07 to 8.58±0.08 g) were obtained from the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State. The *Clarias gariepinus* juveniles were acclimatized for 14 days and fed with 2mm commercial feed twice a day before the commencement of the experiment (Adeyeni *et al*., 2022). Fish were starved for 24 hours before being placed on experimental diets (Adeshina *et al*., 2023). Twenty-one plastic bowls (55L) containing water were used, with three tanks per diet representing triplicate per experimental diet (Bawiec *et al*., 2023). Fifteen *Clarias gariepinus* juveniles were allotted into each of the plastic tanks and were fed twice daily between 08:00-09:00 GMT for mornings and 16:00-17:00 GMT for evenings for 70 days.

**2.5       Growth Indices**

An electrical weighing balance (Model no: XY15KMB) was used to weigh the fish before the experiment started and every two weeks throughout its duration to adjust the feeding level. At the end of the experiment, the weight of the fish was measured to obtain their final weight (Raissy *et al*., 2022).

**2.6       Calculations**

The formulas below were used for the evaluation of growth performance and nutrient utilization among experimental groups (Sahandi & Jafaryan, 2023).

Weight gain (g) = Final body weight ̶ Initial body weight

Specific growth rate (%/day)

Percentage weight gain

Feed Intake (g)

Feed Conversion Ratio

Feed Efficiency Ratio

Protein Efficiency Ratio

Protein Intake

Survival %

**2.7 Proximate Analysis and Statistical Analysis**

The proximate composition of the feed was determined using the standard methods of the Association of Official Analytical Chemists (AOAC) (Hasan *et al*., 2022). All collected data were checked for normality using Levene’s test for homogeneity of variance. A multivariate analysis of variance (MANOVA) was conducted to test for significant differences in the means using the Statistical Package for the Social Sciences (SPSS 22.0 for Windows) (Cheung *et al*., 2024).

**3.0 RESULTS**

**3.1 Mineral Content of Citrus Peels**

The mineral content of the citrus peels is presented in Table 2. The highest Iron (9.4), Calcium (1146), Phosphorus (55), and Potassium (490) were observed in Orange Peels while Tangerine peel had the highest Magnesium (3.6) and Zinc (2.0) content. The highest Sodium (40) content was recorded in the Tangelo peel.

**3.2 Growth Performance and Nutrient Utilization of *Clarias gariepinus* Fed Experimental Diets**

Table 3 presents the growth performance and nutrient utilization of *Clarias gariepinus* fed the experimental diets. The initial mean weight of *C. gariepinus* ranged from 8.48±0.07 to 8.58±0.08 g, with no significant differences observed among the diets. However, mean weight gain (MWG), final mean weight (FMW), specific growth rate (SGR), and survival rate (S) were significantly different (*p* < 0.05). Fish fed the control diet exhibited the highest FMW, MWG, percentage weight gain (PWG), SGR, and survival, with values of 32.37±1.33 g, 23.82±1.29 g, 73.50±0.99%, 1.90±0.05%, and 97.77±2.23%, respectively. Conversely, fish fed the TGR2 diet had the lowest FMW, MWG, PWG, SGR, and survival, with values of 14.23±0.83 g, 5.67±0.86 g, 39.40±3.71%, 0.72±0.09%, and 51.11±2.22%, respectively.

A significant difference (*p* < 0.05) was observed among the treatments for feed intake (FI), feed conversion ratio (FCR), feed efficiency ratio (FER), and protein intake (PI). Feed intake values ranged from 50.91 g to 62.54 g. Fish fed the control diet had the highest FI, FER, and protein efficiency ratio (PER), with values of 62.54±1.49 g, 0.51±0.03, and 0.81±0.03, respectively. The highest FCR (9.13±0.48) was recorded in fish fed the TGR2 diet, while the lowest FCR (1.91±0.01) was observed in fish fed the control diet. The highest PI value (26.21±0.92 g) was recorded in fish fed the ORG2 diet, whereas the lowest PI value (20.38±2.04 g) was observed in fish fed the TGR2 diet.

**3.3 Water Quality Parameters of *Clarias gariepinus* Juveniles**

The water quality of the habitat of Clarias gariepinus juveniles was assessed, and the results are presented in Table 4. No significant differences were observed in temperature, pH, and dissolved oxygen across all treatment groups.

**4.0       DISCUSSION**

The mineral analysis showed potassium and calcium as the most abundant in the peels. Studies affirmed the presence of these minerals in other agro-waste materials. Their roles in enhancing the nutrient quality of produce when utilized for various forms of production have been highlighted (Udeozo *et al*., 2018; Agbagwa *et al*., 2020). The high potassium content in orange peels may be attributed to its natural abundance in orange tissues (Wastowski *et al*., 2013).

Maintaining optimal water quality is essential for ensuring effective growth, survival, overall welfare, and the sustainability of fish rearing (Ehiagbonare & Ogunrinde, 2010). In this Study, temperature readings remained consistent, ranging from 26.47°C to 26.67°C. These values fall within the recommended range of 23°C to 30°C for optimal fish growth, as reported by Yanuhar *et al*. (2021). The pH values recorded in this study ranged from 7.15 to 7.41, aligning with the optimal pH range for catfish growth, as stated by Trisna & Sasanti (2013). Additionally, all dissolved oxygen (DO) levels measured in both the treatment and control groups were within the recommended range, as reported by Rahmawati *et al*. (2021).

The treatments administered to *Clarias gariepinus* juveniles resulted in notable growth improvements; however, the observed growth was significantly different to that of the control group. Fish fed ORG2 had the highest growth performance, with a final mean weight (FMW) of 26.50±0.77 g, mean weight gain (MWG) of 17.92±0.79 g, and percentage weight gain (PWG) of 67.89±1.29%. Elsayed and Salem (2018) reported a significant growth in Nile tilapia-fed diets containing orange peels. The replacement of fish meal with black soldier fly larvae (BSFL) meal in red drum (*Sciaenops ocellatus*) has been linked to reduced growth performance (Yamamoto *et al*. 2022). Florien *et al*. (2022) observed slower growth in Nile tilapia fed a diet containing 75% BSFL meal, whereas higher growth rates were recorded in fish-fed diets containing 25% and 50% BSFL meal. Higher inclusion levels of BSFL meal are believed to suppress feed digestibility and growth rates in farmed fish (Muin & Taufek, 2024). The amino acid profile of black soldier fly (BSF) larvae differs from that of fishmeal in terms of amino acid composition (Mohan *et al*., 2022). However, fishmeal contains higher levels of some amino acids such as taurine, which BSF larvae lack (Ravindran and Blair, 2017). BSF larvae contain relatively lower levels of aromatic amino acids, such as phenylalanine and tyrosine, compared to fishmeal (Oktaviana *et al*., 2022).

BSF larvae serve as a promising sustainable protein source for animal feed, the amino acid profile has to enhance the dietary requirements of some aquaculture species, especially in terms of the conventional highly digestible and balanced profile of fishmeal (Yeganeh *et al*., 2023).

The high mortality of fish in this study align with previous studies by Nairuti *et al*. (2021), which reported that a higher inclusion level of BSF larvae meal (80%) led to increased mortality in Nile tilapia.

**5.0 CONCLUSION**

In this study, the total dietary replacement of fishmeal with black soldier fly (BSF) larvae, contributing 25% of the protein, negatively impacted growth performance and nutrient utilization. Although BSF larvae are rich in protein, they may not be an ideal standalone protein source. However, citrus peels particularly orange peels when incorporated into the diet, can serve as effective additives for promoting sustainable aquaculture feeds. To ensure adequate nutritional intake, BSF larvae should be paired with more complete protein sources.

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**TABLES**

Table 1: Ingredients and Proximate composition of the Experimental Diets (g/100g)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| INGREDIENTS |  | CONTROL | ORG2 | TGR2 | TGL2 | ORG1 | TGR1 | TGL1 |
| Fishmeal (40.25%) |  | 25 |  |  |  |  |  |  |
| BSF (41.1%) |  |  | 25 | 25 | 25 | 25 | 25 | 25 |
| SBM (44%) |  | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| BM (86%) |  | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| GNC (45%) |  | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| Maize (10%) |  | 14 | 14 | 14 | 14 | 14 | 14 | 14 |
| Cassava Flour |  | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Oil |  | 2 | 2 | 2 | 2 | 3 | 3 | 3 |
| ORG 2 |  |  | 2 |  |  |  |  |  |
| TGR 2 |  |  |  | 2 |  |  |  |  |
| TGL 2 |  |  |  |  | 2 |  |  |  |
| ORG 1 |  |  |  |  |  | 1 |  |  |
| TGR 1 |  |  |  |  |  |  | 1 |  |
| TGL 1 |  |  |  |  |  |  |  | 1 |
| VP |  | 2 |  |  |  |  |  |  |
| TOTAL  **Proximate Composition** |  | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Moisture Content |  | 8.84 | 8.59 | 8.99 | 8.00 | 8.45 | 8.12 | 8.71 |
| Crude Protein |  | 39.87 | 39.05 | 40.03 | 39.91 | 39.61 | 39.95 | 38.21 |
| Crude llipid |  | 13.08 | 12.98 | 13.22 | 13.34 | 12.86 | 13.85 | 12.93 |
| Crude fibre |  | 4.52 | 5.01 | 5.73 | 4.91 | 4.97 | 5.12 | 4.88 |
| Ash |  | 11.44 | 11.28 | 10.84 | 13.72 | 15.01 | 13.90 | 12.88 |
| NFE |  | 22.75 | 23.09 | 21.19 | 21.12 | 19.10 | 19.06 | 22.39 |

Premix: Vitamin A; 10,000,000.00I.U, Vitamin D3; 2,000,000.00 I.U, Vitamin E; 23,000.00mg Vitamin K3; 2,000.00mg, Vitamin B1; 3,000.00mmg, Vitamin B2; 6,000.00mg, Niacin; 50,000.00mg, Calcium Pantothenate; 10,000.00; Vitamin B6; 5,000.00mg, Vitamin B12; 25.00mg, Folic Acid; 1,000.00mg, Biotin; 50.00mg, Choline Chloride; 400,000.00mg, Manganese; 120,000.00mg, Iron; 100,000.00mg, Zinc; 80,000.00mg, Iodine; 1,500.00mg, Cobalt; 300.00mg, Selenium; 120.00mg, Anti-oxidant; 120,000.00mg. (Source: Chemiconsult International Limited, Ikeji, Lagos, Nigeria). Recommended Inclusion Rate is 2.5kg per tonne of final feed. NFE: Nitrogen Free Extract, VP: Vitamin-mineral premix;

**Table 2. Minerals content (mg/100g) of citrus peels (dry weight basis)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Orange | Tangerine | Tangelo |
| Magnesium | 2.9 | 3.6 | 2.5 |
| Sodium | 36 | 38 | 40 |
| Iron(Fe) | 9.4 | 8.2 | 9.2 |
| Zinc | 1.9 | 2.0 | 1.8 |
| Calcium | 1146 | 865 | 913 |
| Phosphorus | 55 | 48 | 50 |
| Potassium | 490 | 456 | 487 |

**Table 3. Growth Performance, Nutrient Utilization and Survival of *Clarias gariepinus* Fed Experimental Diets**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | Control | ORG2 | TGR2 | TGL2 | ORG1 | TGR1 | TGL1 |
| IMW(g) | 8.55±0.60a | 8.58±0.08a | 8.56±0.06a | 8.48±0.07a | 8.57±0.05a | 8.55±0.08a | 8.58±0.04a |
| FMW(g) | 32.37±1.33e | 26.50±0.77d | 14.23±0.83a | 18.59±1.03bc | 21.00±0.40c | 16.71±0.92ab | 17.55±0.36b |
| MWG(g) | 23.82±1.29e | 17.92±0.79d | 5.67±0.86a | 10.10±1.04bc | 12.43±0.41c | 8.16±0.95ab | 8.96±0.34b |
| PWG (%) | 73.50±0.99d | 67.89±1.29d | 39.40±3.71a | 54.06±2.71bc | 59.15±0.87c | 48.49±3.16b | 51.05±0.92b |
| SGR | 1.90±0.05e | 1.61±0.05d | 0.72±0.09a | 1.13±0.09bc | 1.28±0.03c | 0.95±0.08b | 1.02±0.03b |
| FI(g) | 62.54±1.49a | 60.45±9.02a | 50.91±5.09a | 52.76±1.26a | 52.97±2.01a | 56.38±2.33a | 56.94±2.46a |
| FCR | 1.91±0.01a | 3.77±0.27b | 9.13±0.48d | 5.36±0.70c | 4.13±0.08b | 6.12±0.06c | 6.38±0.40c |
| FER | 0.51±0.03e | 0.27±0.02d | 0.11±0.01a | 0.19±0.02bc | 0.24±0.01cd | 0.14±0.02ab | 0.16±0.01ab |
| PER | 0.81±0.03e | 0.49±0.02d | 0.18±0.00a | 0.27±0.01b | 0.32±0.01c | 0.25±0.00b | 0.23±0.00b |
| PI | 24.94±0.60c | 26.21±0.92cd | 20.38**±**2.04a | 21.05±0.50b | 22.38±1.00bc | 22.52±0.93bc | 21.75±0.94bc |
| S (%) | 97.77±2.23e | 86.67±0.00d | 51.11±2.22a | 73.33±3.85c | 55.55±2.22a | 53.33±0.00a | 66.67±0.00b |

Mean values with the same alphabet in superscript along the column are not significantly difference (p>0.05) from one another. IMW (Initial Mean Weight), FMW (Final Mean Weight), MWG (Mean Weight Gain), PWG (Percentage Weight Gain), SGR (Specific Growth Rate)

**Table 4: Water Quality Parameters of *Clarias gariepinus* Juveniles**

Values are means ± SE. Mean values with the same alphabet in superscript along the column are not significantly difference (p>0.05) from one another using the Duncan Multiple Range Test.

|  |  |  |  |
| --- | --- | --- | --- |
| Treatments | TEMP(0C) | pH | DO(mg/l) |
| ORG1 | 26.60±0.58a | 7.15±0.01a | 5.43±0.03a |
| TGR1 | 26.50±0.00a | 7.15±0.05a | 5.40±0.06a |
| TGL1 | 26.47±0.88a | 7.18±0.03a | 5.63±0.09a |
| ORG2 | 26.47±0.67a | 7.38±0.01a | 5.07±0.12a |
| TGR2 | 26.47±0.15a | 7.41±0.00a | 5.80±0.00a |
| TGL2 | 26.47±0.15a | 7.35±0.01a | 5.17±0.09a |
| CONTROL | 26.67±1.20a | 7.41±0.03a | 5.67±0.03a |