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Co-incorporation of Black Soldier Fly (*Hermetia illucens*) Larvae and Moringa (*Moringa oleifera*) Leaves in the Diet Increase African Catfish Profitability (*Clarias gariepinus*)

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Abstract

Aquaculture is a fast-growing activity in Cameroon and required substantial quantities of affordable protein-rich feed. In this study, we co-incorporated *Moringa* leaves (MOL) and black soldier fly larvae meal (BSFLM) in catfish diet, and the effect was evaluated on feed floatation, fish growth, flesh organoleptic qualities and profitability. 450 juvenile's catfish weighing averagely 10 g, were subjected to five dietary treatments consisting of: T0 (0% BSFLM and 0% MOL); T1 (6.25% BSFLM and 5% MOL); T2 (12.5% BSFLM and 10% MOL); T3 (18.75% BSFLM and 15% MOL) and T4 (25% BSFLM and 20% MOL) for 119 days. The treatment T3 containing 18.75% BSFLM and 15% MOL recorded the highest floatation duration, fish weight (113.83±17.31 g) and weight gain (103.25±17.8 g), and the lowest feed conversion ratio (1.60±0.37). The flesh tasted good and was acceptable in all treatments. Incorporating BSFLM and MOL in the diets reduced the cost per kg of feed from 18 to 23%, thus increasing the system's profitability from 45.91 to 60.84%. We concluded that BSFLM and MOL can partially substitute fish meal in catfish diet and improve the profitability and offers insights into the development more profitable and sustainable aquaculture in Cameroon and globally.

Introduction

Cameroon faces a deficit in fish products to the extent that it heavily relies on fish imports (INS, 2019). Indeed, fish demand is estimated at 400,000 tons per year with only 289,764 tons/year as domestic production (MINEPIA, 2019), which remains significantly below demand. To ensure the availability of this resource, the Cameroonian government annually spends over 154 Million Euros on frozen fish imports (MINEPIA-DEPCS., 2020. Thus, to reduce these imports, which not only create a dependence on the outside (FAO, 2014) and a deficit on the country's trade balance, the government has decided to support entrepreneurs willing to engage in aquaculture (Ecomatin, 2020), through the popularization of fish farming, with the main species being *Clarias gariepinus*, commonly known as catfish. African catfish has zootechnical advantages such as hardiness, diversified diet, and highly appreciated flesh (Więcaszek *et al*., 2010; Ahotondji, 2012). Although local entrepreneurs are motivated to raise it, they face a myriad of challenges, among which is feeding. Indeed, the cost of feed represents 40 to 60% of production costs in intensive farming systems, and the economic viability of the farming operation depends on the quality of the feed (Bamidele *et al*., 2015; Nyadjeu et *al*., 2020). Moreover, Mkong *et al.* (2018) found that in Cameroon fish farming profitability mostly influenced by the price of feed. Due to the high cost of imported feeds and the unavailability of certain ingredients such as the protein component, mainly consisting of fish meal and soybean meal, which are the most expensive (Alphonsus *et al*., 2009; Newkirk, 2010), it becomes urgent to find alternative, available, and less expensive protein sources. Thus, several studies have been conducted on replacing fish meal with other protein sources, whether of plant or animal origin (Olaniyi and Salau, 2013; Miégoué *et al*., 2018). Fish meal is generally the major component of aquaculture feeds. However, the sustainability of the fish industry depends on reducing the use of fish in fish feed production (Francis *et al*., 2001). This study was therefore oriented towards the use of insect meal, which has seen a growing trend in recent years as a sustainable protein source in aquatic animal feed production (Weththssinghe *et al*., 2021). Therefore, the black soldier fly larvae (BSFL), scientifically known as *Hermetia illucens*, was studied for its ability to convert organic waste into high-quality protein (Baragan *et al*., 2017). Moreover, vegetable protein sources have gained significant importance among aquaculturists and nutritionists due to their sustainability, environmental safety, and relatively lower price. Among these, *Moringa oleifera* stands out for its high nutritional properties, especially in its leaves (Moringa new, 2022). Currently, numerous studies have been conducted on the use of black soldier fly meal (Maina, 2020) and Moringa leaf meal (MOL) (Ekelemu *et al*., 2023) as protein sources in the diet of African catfish, revealing their improvement potential on growth performance and other zootchnical characteristics (Talamuk, 2016). However, most growth experiments conducted on various species, including African catfish, show that inclusion rates of insect meals in the diet averaging 25% do not affect fish growth and size. Beyond these levels, issues of digestibility, slow growth, and fertility are raised (Talamuk, 2016; Cacot *et al*., 2021). Meanwhile, Nsofor *et al*. (2012) demonstrated that *Moringa oleifera* leaves can be included up to 20% in the diets of African catfish. However, the synergistic effect of the combination of the two flours could make it possible, to increase their incorporation rates into the feed beyond those reported by Talamuk (2016) with regard to the black soldier fly and by Nsofor *et al.* (2012) regarding Moringa leaves. In other hand; these local available feed resources will make fish production more cost effective and sustainable as it is produced with little foot-spring. Moreover, the binding capacity on BSFL fat and lower density of MOL can improve feed buoyancy thereby reducing the consumption index. Flotability is very important in fish it enable the farmer to observe the feeding activity of the fish, thus prevent wastage of feed (Wan *et al*., 2023). Additionally, the combination can enhance growth performance of *Clarias gariepinus* due the synergistic effect of actives compound found in MOL and BSFL. Nevertheless, to the best of our knowledge, no research has been conducted on the combined utilization of these two resources in fish feed. In order to address the aforementioned gaps, this study was initiated with the objective of evaluating the effects of combining *Hermetia illucens* meal and *Moringa oleifera* leaf meal, and their respective inclusion rates in the diet, on the feed buoyancy, survival, growth performance, organoleptic qualities of fish flesh and production costs in *Clarias gariepinus*.

Materials and Methods

Study Area

The study was conducted at the Entomoculture unit of the Faculty of Agronomy and Agricultural Sciences, Annex of Bafia, located in the Centre Region, Mbam and Inoubou Division, specifically in the Bafia Sub-division, between 4°37' and 4°46' North latitude and 11°6' and 11°18' East longitude. This area is at an average altitude of 495 meters above sea level (PCD Bafia, 2015). The climate in Bafia is equatorial and includes a rainy season (mid-March to mid-November) and a dry season (mid-November to mid-March). The average temperature and rainfall are 25°C and 1250 mm/year, respectively.

Animal Materials

Fingerlings

For this study, 450 *Clarias gariepinus* fingerlings with an average weight of 10 ± 2 g and average total and standard lengths of 10.5 ± 1.75 cm and 8.9 ± 2.22 cm, respectively were used.

Black Soldier Fly Larvae

Black soldier fly larvae were produced at the entomology unit of the Faculty of Agronomy and Agricultural Sciences, Annex of Bafia. Young colonies of larvae aged 7 days and permanently maintained at the Bafia entomology unit since 2021 were used. Their feeding was made from organic waste collected from restaurants in the city of Bafia. These organic wastes were mainly made up of starchy peelings, fish viscera, vegetables and fruits, chicken and beef wastes (viscera). For their production (15 days), the *Hermetia illucens* larvae were placed in the substrate consisting of chick starter food in the form of prepulp, which became the flies. The flies' eggs were collected and then incubated to obtain the larvae as described by Dzepe *et al*. (2019). Once produced, they were killed in boiled water at 50°C for 30 minutes. The dead larvae were sun-dried to a constant weight and ground using a manual machine to obtain black soldier fly larvae meal (BSFLM). This meal was analyzed (Organic matter: 69.77%; crude protein: 42.30%; fat: 25.08% and ash: 12.11%) in the laboratory of Animal Nutrition, University of Dschang, then stored away from moisture during use.

Moringa Leaves

Fresh Moringa leaves (MOL) were harvested from the surrounding plantations, dried in the shade at room temperature on paper sheets. After dehydration, they were ground using a mill to obtain the powder (Moringa meal). The powder obtained where analyzed for proximate composition (Organic matter: 69.40%; crude protein: 25.40%; fat: 1.09% and ash: 7.05%) in the laboratory of Animal Nutrition, University of Dschang. Thereafter, it was used in feed formulation.

Housing and Equipment

The fish were raised in circular tanks of 125 liters each with a useful volume of 110 liters, placed on flooring 0.5 m high installed inside a room. Each tank was equipped with a water feeding device and an overflow pipe with a 32 mm pressure tube, which also served as a drain outlet and maintained a constant water level in the tanks. At the bottom of each tank, a circular basin with a diameter of 30 cm was installed to collect leftovers. These basins were placed vertically under a circular floating frame (feeder) with a diameter of 28 cm, installed on the surface of the water in each tank. The breeding tanks were labeled according to the different treatments and covered with a net to prevent the fish from escaping.

Diets Formulation and Experimental Design

A control diet T0, not containing black soldier fly meal or Moringa leaves was formulated locally. On the basis of the latter, four other diets: T1, T2, T3, and T4 containing BSFLM and Moringa were formulated at respective levels of 25%, 50%, 75%, and 100% (based on the incorporation limits of black soldier fly meal and Moringa leaves) of black soldier fly meal and *Moringa oleifera* meal. The ingredients were finely ground using a Moulinex and sieved before being incorporated into the mixture. These different diets were iso-proteinic. After formulation, water (2 liters for 10 kg of feed) was added to obtain a paste. The formulated and moistened feeds were granulated using a 2 mm diameter mesh granulator and then dried in the sun until a constant weight.

Each of the 5 experimental diets (Table 1) was randomly assigned to 5 experimental units in a completely randomized design.

Data Collection and Study Parameters

Floatability Study

The floatability assessment was done according to Maguelong (2021) protocol. This method involved taking twenty (20) seeds successively in each treatment three times, then pouring them into a container of water and using a chronometer to determine the time and the number of seeds at the beginning and end of immersion.

Growth Characteristics

The study took place in 18 plastic tanks as previously described, in which 450 *C. gariepinus* fingerlings were randomly distributed into six comparable groups with three (3) repetitions per groups. Each tank contained 25 individuals and water was renewed daily to two-thirds (2/3) capacity. Each group was randomly assigned one of the experimental rations T0; T1; T2; T3; and T4 formulated earlier. The animals were fed twice a day at fixed times (7-8 a.m. and 5-6 p.m.) at 5% of their fish biomass for 17 weeks (119 days)). The feed was adjusted after each control fishing. Feed leftover was also collected every two days using a basin placed at the bottom of the tanks. These collected feed were dried in the sun to a constant weight, then weighed to estimate feed intake. A control fishing was carried out every two weeks on 50% of the total fish population in each tank. These fish were weighed individually using a precision electronic scale and measured (total and standard length) using a 1 mm

Table 1: Centesimal composition of experimental diets

	Rations				
Ingredients	T0	T1	T2	T3	T4
Maize	7	10	7	4	2
Cassava	8	8	6	4	2
Screenings	13	11	6	3.75	2
Soybean cake	18	14	10	7	5
Peanut cake	3	5.75	9.50	10	14
Palm kernel cake	1	2	5	8	7
BSFLM	/	6.25	12.5	18.75	25
Blood meal	10	11	11	13.50	11
MOL	/	5	10	15	20
Fish meal	28	24	20	16	10
Oil	2	1	1	0.50	/
Premix 10%	2	2	2	2	2
Total	100	100	100	100	100
Crude protein %	40,56	40.44	40.69	40.43	40.41
ME (kcal)	3087.16	3068.62	3089.26	3083.03	3161.21

precision ichthyometer to evaluate growth characteristics and adjust the amount of feed to be distributed for the next 14 days. At the same time, while collecting the data, the physico-chemical characteristics of the water were measured on-site between 6 and 7 a.m. in each tank twice a week according to the Rodier *et al*. (2009) protocol. This involved taking the water temperature and pH using an HTC2 thermometer and a pH meter respectively. At the end of the trial, all fish were counted, weighed, and measured.

Data Collection and Studied Parameters

Survival Rate

The survival rate refers to the proportion of fish still present in the tanks at the end of the experimental period compared to the total number of fish at the beginning. It was calculated as follows:

$$\text{Survival rate (\%)} = \frac{(\text{Final live count})\text{X}100}{\text{Initial fish count}}$$

Feed Consumption

Food consumption (CA) was calculated by taking the difference between the amount of feed distributed and the leftover collected at the end of the same period.

Feed Consumption (g)=Amount of feed served (g)-leftover (g)

Total and Standard Length Gain

Length gain (cm)=Final length-Initial length

Final Average Weight

It refers to the weight of the fish at the end of the experiment.

Weight Gain

Weight gain= final weight – initial weight

Specific Growth Rate

$$\text{Specific growth rate (\% per day)}=\frac{(\ln \text{final weight} - \ln \text{initial weight})\text{ X }100}{\text{Time (number of days)}}$$

With ln = natural logarithm

Condition Factor K

$$\text{Condition factor (K)}=\left(\frac{\text{Live weight}}{(\text{Total length})^3}\right)\text{ x }100$$

Feed Conversion Ratio

$$\text{Consumption index}=\frac{\text{feed intake}}{\text{final weight} - \text{initial weight}}$$

Stomatic Index Evaluation

Stomach indices are parameters used to evaluate the effect of diet on the internal organs and conditions of fish. The methodology described by Piccolo *et al*. (2017) was used to analyze these indices which included:

$$\text{Indice Viscéraux Somatique (IVS (\%)}=\left(\frac{\text{visceral weight (g)}}{(\text{body weight(g)}}\right)\text{x}100$$

$$\text{Hepato-somatic Index (HSI (\%)}=\left(\frac{\text{liver weight (g)}}{(\text{body weight (g)}}\right)$$

Evaluation of Economic Sustainability

Raw growth performance data and feed utilization parameters allowed us to evaluate the economic parameters to provide enough data for statistical evaluation. Three replicates were used for each treatment according to the method used by Rawski *et al*. (2021). For each treatment, Feed Conversion Efficiency (FCE) was used as a growth index to obtain a Feed Conversion Ratio (FCR) providing information on the amount of fish gain for each feed. Sunde *et al*. (2004) used the following formula:

$$\text{Feed Conversion Efficiency (FCE)}=\frac{\text{Body weight gain (g)}}{\text{Feed intake (g)}}$$

The value of fishmeal utilization can be defined as the proportion of fishmeal found in the feed multiplied by the feed conversion index (Gasco *et al*., 2018).

Fishmeal Utilization for Fish Production (g/1 kg of fish gain) = fishmeal proportion in the feed (g/1 kg) × Feed Conversion Index

The Fish In/Fish Out Ratio (FIFO)

The fish in/fish out ratio was determined according to Rawski *et al*. (2021) as the amount of live fish from capture fisheries needed for each unit of farmed fish produced. It is calculated as follows:

FIFO = [(level of fishmeal in the feed (g/1 kg) + level of fish oil in the feed (g/1 kg))/ (yield of fishmeal from wild fish (g/1 kg) + yield of fish oil from wild fish (g/1 kg))] × (feed intake (g)/body weight gain (g))

To achieve environmentally sustainable levels, the FIFO value should be ≤ 1, meaning it does not result in a fish harvest exceeding production levels (Tracon *et al.*, 2012).

Relative Efficiency and Economic Advantage

This is determined through the Economic Conversion Rate (ECR) and the Economic Profit Index (EPI), which were calculated using the formulas of Stejskal *et al*. (2020).

$$\text{ECR (Euro/1 kg of fish gain)} = \left(\frac{\text{feed intake (g)}}{\text{body weight gain(g)}}\right) \text{x feed cost (Euro/1 kg)}$$ (Van Huis, 2017).

EPI (Euro/fish) = [body weight gain (kg) × live fish selling price (Euro/1 kg)] – [body weight gain (kg) × (feed cost (Euro/1 kg) × (feed intake (g)/body weight gain (g)] (IPIFF, 2021).

Profitability

Profitability (R) was calculated based on the balance between the selling price of the live fish and the feeding costs per kilogram of fish gain (ECR). The calculation was done using the following formula:

R (EURO/1 kg of fish gain) = live fish selling price (Euro/1 kg) - Economic Conversion Rate (Euro/1 kg of fish gain) (Huang *et al.,* 2019).

Other fish production costs were not included.

Evaluation of Organoleptic Characteristics of Fish Carcasses

The evaluated organoleptic characteristics included color, tenderness, flavor, juiciness, smell, taste, and acceptability of the flesh (Adibe *et al*., 2018). They were assessed using sensory methods involving a group of students selected based on their food preferences (non-alcoholic, non-smokers). They were trained to distinguish between different organoleptic parameters.

Preparation of Broth for Tasters Selection

Unsalted and unseasoned fish broth was prepared by cooking 250g of fish in 1/2 liter of drinking water for 20 minutes over low heat. Then, 25 ml of this broth was diluted with 1 liter of mineral water. Before dilution, the broth was cooled to room temperature, filtered, and diluted with 1 liter of water to obtain a solution that appeared identical to water. This liquid was used to select tasters through the triangular method. Selected tasters were briefed on the evaluation procedures and expectations.

Preparation of Fish Samples

Different fish samples (250g/portion) without water or seasoning were wrapped in aluminum foil, placed on a stainless steel plate, and cooked with 1/4 liter of water in a pot so that the fish did not come into contact with the water. These samples were cooked for 20 minutes over low heat.

Tasters Selection

Tasters were selected based on their ability to distinguish three very similar samples using a simple difference test (triangular test). This test involved identifying the taste difference among three cups or coded samples presented simultaneously to the taster, two of which were identical (containing only mineral water) while the third contained previously diluted fish broth (dilution at 2.5%). The panelist was asked to identify the different sample. Those who identified the cup containing diluted fish broth were selected to evaluate the organoleptic quality. For this study, 35 tasters were selected and briefed on the evaluation procedures and expectations.

Tasting and Evaluation of Organoleptic Quality of Fish Carcasses

Tasting took place in one session in a room. Selected tasters were initially trained. This training involved explaining the meaning of each technical term (color, tenderness, flavor, etc.). They proceeded to taste the cooked fish from the different portions. Tasters consumed bread and drank water between successive tastings to eliminate the taste from the previous evaluation. After each tasting, they were asked to assign a score ranging from 1 to 8 based on various criteria for evaluating the level of preference for the fish carcass. Scores were allocated using an 8-point hedonic scale (Okeke *et al*., 2022).

Statistical Analysis

The data on floatability, survival, growth parameters, production costs, and organoleptic characteristics of fish carcass were subjected to one-way analysis of variance (ANOVA). Regression tests were used to establish relationships between the levels of incorporation of black soldier fly meal and Moringa leaf meal and growth parameters. When there were significant differences between the means, they were separated by the Fisher test at a significance level of 5%. The statistical software SPSS 20.0 (Statistical Package for Social Sciences) was used for these analyses.

Results

Effects of Incorporating Black Soldier Fly Larvae Meal and Moringa Leaves on the Feed Buoyancy

Table 2 summarizes the effect of incorporating black soldier fly larvae meal (BSFLM) and *Moringa olifera* leaves (MOL) on the buoyancy of the feed. It emerges that the highest floating duration ($P<0.05$) was recorded with diet T3 containing 18.75% BSFLM and 15% MOL as compared to the remaining batches.

Effects of Black Soldier Fly Larvae Meal and Moringa Leaves on the Growth Parameters of African Catfish

Globally, all the growth characteristics were significantly affected ($P<0.05$) by the incorporation of BSFLM and MOL in the diet except for the K factor, which remained comparable across all diets (Table 3). In addition, the fish growth characteristics tend to increase with the level of BSFLM and MOL in the diet up to T4 when it starts decreasing. Then, the highest values (P<0.05) were recorded in fish subjected to the T3 diet, except for the feed conversion ratio, which actually obtained the lowest value. Reversely, the lowest growth parameters values were obtained with fishes fed on diets T1 and T3.

Feed Intake

As presented in Table 3, there was an increase in consumption with the increasing incorporation of BSFLM and MOL, with the highest values recorded for fish in treatments T4 (182.51±2.45 g) and T3 (173.77±6.15 g), which were comparable to each other and significantly higher than other diets. Reversely, the lowest feed consumption value (71.70±9.62 g) was observed in fishes fed on the diet without BSFLM and MOL (T0).

Live Weight and Weight Gain

As well as feed intake, there was an increase in live weight with the incorporation of BSFLM and MOL in the diet up to treatment T4, where a decrease was observed. Therefore, the average live weight and weight gain of fish receiving the diet T3 were significantly higher (P<0.05) than those of the others diets. In contrary, the lowest fish weight was recorded in diets T1 and T2.

Total and Standard Length

The significantly highest average total and standard lengths were recorded in fish fed diet T3 and T4 as compared to the rests of the treatments (Table 3).

Feed Conversion Ratio

As shown in Table 3, the feed conversion ratio tends to decrease with the increase in the incorporation rate of MOL and BSFLM in the diet. Thus, the T3 with 18.75% BSFLM and 15% MOL recorded the lowest feed conversion ratio among all groups, however comparable (P<0.05) to that of the control group (T0). Reversely, the highest Feed conversion ratio (5.45±0.58) was obtained with the T1 diet.

Specific Growth Rate

The specific growth rate was significantly affected by the level of incorporation of MOL and BSFLM in the diet (Table 3). The specific growth rate of fish fed with T3 diet was significantly higher (P<0.05) as compared to those of the other treatments.

Table 2: Effects of the level of incorporation of black soldier fly larvae meal and Moringa leaves on the buoyancy of the feed

	Diets					p
Treatments	T0	T1	T2	T3	T4	
Number of seeds at the beginning of immersion	14.75±4.57[b]	14.75±4.34[b]	10.25±3.09[ab]	6±3.91[a]	11.25±3.86[ab]	0.04
Number of seeds at the end of immersion	5.25±4.57[a]	5.25±4.34[a]	9.75±3.09[ab]	14±3.91[c]	8.75±3.86[ab]	0.04
Duration of the beginning immersion (s)	3.00±2.00[a]	2.75±0.50[a]	2.75±0.50[a]	3.00±0.00[a]	3.25±0.500[a]	0.94
Duration of flotation (s)	338.33±180.99[a]	330.75±101.73[a]	488.00±86.213[a]	666.00±117.61[b]	462.75±15.52[a]	0.00

a,b and c: means bearing the same letters in the same line are not significantly different (P>0.05). P= Probability, T0: control diet locally formulated without black soldier fly larvae flour or Moringa leaves; T1: Diet containing 6.25% black soldier fly larvae flour and 5% Moringa leaves; T2: Diet containing 12.5% black soldier fly larvae flour and 10% Moringa leaves; T3: Diet containing 18.75% black soldier fly larvae flour and 15% Moringa leaves; T4: Diet containing 25% black soldier fly larvae flour and 20% Moringa leaves.

Table 3: Effects of incorporating black soldier fly larvae meal and Moringa leaves on the growth performance of African catfish (*Clarias gariepinus*)

	Diets					
Characteristics	T0	T1	T2	T3	T4	p
SR (%)	68.75 ±28.64[a]	75.00 ±25.00[a]	72.92 ±3.61[a]	81.25±5.46[b]	93.75 ±10.83[bc]	0.28
FI (g)	71.70±9.62[a]	84.26±6.14[b]	92.78±21.68[b]	173.77±6.15[c]	182.51±2.45[c]	0.00
Final live weight (g)	50.10±2.77[b]	27.18±0.40[a]	29.33±0.49[a]	113.83±17.31[d]	68.66±2.46[b]	0.00
FTL (cm)	16.71±1.75[b]	14.03±0.85[a]	14.14±7.72[a]	24.24± 3.28[c]	23.53±3.29[c]	0.00
FSL (cm)	1.95±1.63[a]	12.73±0.99[a]	12.7±1.82[a]	22.22±1.76[b]	21.24±12.61[b]	0.00
WG(g)	40.10±2.28[b]	16.66±1.73[a]	19.33±1.44[a]	103.25±17.8[d]	58.06±2.24[c]	0.00
FCR	1.80±0.25[b]	5.45±0.58[e]	4.74±1.16[d]	1.60±0.37[b]	3.10±0.20[c]	0.00
SGR (%/day)	1.87±0.27[b]	1.04±0.21[a]	1.11±0.14[a]	2.48±0.26[c]	1.87±0.11[b]	0.00
Condition K	1.03±0.38[a]	0.89±0.17[a]	1.02±0.13[a]	1.02±0.06[a]	0.76±0.24[a]	0.49

a, b, c, d, and e: the averages bearing the same letters in the same line are not significantly different. (P>0.05). P= Probability, SR: survival rate FC= Feed intake, FTL= Final total length, FSL= Final Standard length, WG= Weight gain, FCR= feed conversion ratio, SGR= Specific Growth Rate, K= Condition Factor; T0: control diet locally formulated without black soldier fly larvae flour or Moringa leaves; T1: Diet containing 6.25% black soldier fly larvae flour and 5% Moringa leaves; T2: Diet containing 12.5% black soldier fly larvae flour and 10% Moringa leaves; T3: Diet containing 18.75% black soldier fly larvae flour and 15% Moringa leaves; T4: Diet containing 25% black soldier fly larvae flour and 20% Moringa leaves

Condition Factor K

The K condition factor was comparable among the treatments at the end of the study, although this characteristic was highest with the control diet T0 (diet BSFLM and MOL) and the lowest value (0.76±0.24) was observed with treatment T4.

Stomach Indices

It appears from Table 4 that no significant difference was recorded (P>0.05) on the fishes visceral and hepato-somatic indices of the different experimental diets. However, treatment T2 obtained the highest viscera-somatic index (414.95±392.19) and the lowest (208.51±42.77) was observed in fish fed with the diet containing 25% BSFLM and 20% MOL (T4).

Effects of the Incorporation Levels of Black Soldier Fly Meal and Moringa Leaves on the Organoleptic Properties of the African Catfish Flesh

The average values of the organoleptic characteristics of fish flesh subjected to different incorporation rates of BSFLM and MOL in the feed are presented in Table 5. Generally, it appears that the values of organoleptic characteristics were comparable among the treatments except for flavor and taste, which showed significant differences among the treatments. The highest scores for flavor and taste were recorded with the fish from treatments T3 (5.80±1.92) and T4 (5.70±2.56) respectively for flavor and taste.

Effects of Incorporating Black Soldier Fly Larvae on Economic Variable and Sustainability

Table 6 summarizes the effects of incorporating Moringa leaves and BSFLM on the profitability and sustainability of fish farming. From this table, it appears that the feed conversion efficiency was significantly lower (P<0.05) with the T1. Meanwhile, the price per kilogram of feed was higher with the control diet T0. Profitability was significantly lower (P<0.05) with the control treatment T0 and higher with the T1 feed. The most sustainable treatment was recorded with diets T2 to T4 with FIFO value near to 1.

Discussion

Many studies have been carried out on the effect of black soldier fly larvae meal and moringa leaves added separately in the feed in many fish species. However, there are limited studies on the combined effect of the two ingredients in the same feed on the survival rate and growth characteristics of fish. The aim of this study was to formulate diet with local protein sources in order to increase fish farmer sustainability and profitability.

Feed characteristics, particularly feed floatability is very important for farmer it affects the feeding activity of the fish thus preventing wastage of feed. They also exhibit superior characters such as greater water stability, digestibility, water protection, less water pollution and wastage of raw materials (Almaraaj, 2010), in addition, it supplies higher energy than sinking feed. In this study, the effect of black soldier fly larvae meal and moringa leaves in the diet significantly affected the floating capacity. The highest buoyancy duration (666.00 seconds) was recorded with the T3 diet. These results differ from those obtained by Maguelong (2021), who obtained a value of 1798 seconds by incorporating baking powder into the diets. This difference may be due on one hand to the absence of baking powder in our experimental diets; given that, baking it is a feed additive that under certain conditions releases carbon dioxide gas, making the feed lighter (Makoumo, 2021). On the other hand, the latter might be as a result of the quality of the ingredients used in the present study, as the floating duration could be affected due to the density of the different ingredients used in the feed. Globally, the lack of banking agent in this study can be an explicative fact to the low floating duration recorded.

The physicochemical characteristics of the water, especially temperature (21.51°C) and pH (6.76), are within the recommended standards for the survival and growth rates of *Clarias gariepinus*. The survival rate, which is the fish ability to live under farming conditions (Cacot, 2006), increased with the dietary incorporation of MOL and BSFLM. The T4 diet containing the highest rate of MOL and BSFLM recorded the highest survival rate as compared to all the treatment except T3.

The decrease in mortality rates observed in this study seems to be linked to the low stocking density applied to minimize cannibalism and to feeding practices. In addition, Moringa leaves contain a wide range of nutritional value and act as both feed and medicine, thereby improving the survival rate. Independently of the treatment, the survival rates recorded within this trial (68.75 - 93.75%) are higher than those obtained by Phanindra (2005) and Madhav *et al*. (2006), which were 54.9% and 21.33%, respectively, under the same rearing conditions.

Feed consumption could be considered as the amount of feed ingested by the fish within a given time interval. In the present study, the feed consumption increased with the level of MOL and BSFLM in the diet. Therefore, treatment T4 containing the highest incorporation rate in these ingredients recorded the highest feed intake as compared to the rest

The final average live weight and weight gain of *C. gariepinus* were higher with the T3 diet containing 18.75% of BSFLM and 15% of Moringa. The good performance with this diet may be partly related to the very high buoyancy duration recorded in this diet compared to the others. But also by the presence of secondary metabolites in Moringa with a positive effect

Table 4: Visceral and hepato-somatic indices of the different experimental diets

Indices	Diets					p
	T0	T1	T2	T3	T4	
IIVS	400.66±100.47ᵃ	287.39±93.14ᵃ	414.95±19.219ᵃ	198.36±26.54ᵃ	208.51±42.77ᵃ	0.46
IISH	0.01±0.01ᵃ	0.01±0.00ᵃ	0.01±0.00ᵃ	0.00±0.00ᵃ	0.00±0.00ᵃ	0.43

a: the averages bearing the same letters on the same line are not significantly different. (P>0.05). P=Probability, Ivs: visceral stomatal index; iish: hepato-stomatal index T0: control diet locally formulated without black soldier fly larvae flour or Moringa leaves; T1: Diet containing 6.25% black soldier fly larvae flour and 5% Moringa leaves; T2: Diet containing 12.5% black soldier fly larvae flour and 10% Moringa leaves; T3: Diet containing 18.75% black soldier fly larvae flour and 15% Moringa leaves; T4: Diet containing 25% black soldier fly larvae flour and 20% Moringa leaves.

Table 5: Organoleptic characteristics of fish carcasses according to the incorporation levels of BSFLM and MOF in the diet

Characteristics	Diets					p
	T0	T1	T2	T3	T4	
Colour	5.67±2.00ᵃ	6.43±1.31ᵃ	6.40±1.40ᵃ	6.00±1.83ᵃ	6.01±1.75ᵃ	0.49
Tenderness	5.75±1.67ᵃ	5.36±1.30ᵃ	5.57±0.97ᵃ	5.74±2.30ᵃ	5.81±1.61ᵃ	0.29
flavour	3.98±2.40ᵇ	4.09±2.05ᵇ	5.04±1.39ᵃ	5.80±1.92ᵃ	5.04±2.00ᵃ	0.00
Juiciness	5.97±1.46ᵃ	6.57±0.95ᵃ	6.40±1.34ᵃ	5.43±2.30ᵃ	5.10±2.30ᵃ	0.40
Odour	4.48±2.17ᵃ	4.57±2.12ᵃ	4.58±2.20ᵃ	4.80±1.90ᵃ	4.56±2.21ᵃ	0.20
Taste	4.78±2.08ᵇ	4.98±2.60ᵃᵇ	5.68±1.71ᵃ	5.58±1.86ᵃ	5.70±2.56ᵃ	0.01
Acceptability	5.49±1.90ᵃ	5.65±2.02ᵃ	5.96±1.52ᵃ	5.48±1.65ᵃ	5.50±2.01ᵃ	0.28

The scores of organoleptic parameters range from 1 (dislikes extremely) to 8 (likes extremely); a, b, and c: the averages with the same lowercase letters on the same line are not significantly different (P>0.05). P=Probability, T0: control feed locally formulated without black soldier fly larvae flour or Moringa leaves; T1: Feed containing 6.25% black soldier fly larvae flour and 5% Moringa leaves; T2: Feed containing 12.5% black soldier fly larvae flour and 10% Moringa leaves; T3: Feed containing 18.75% black soldier fly larvae flour and 15% Moringa leaves; T4: Feed containing 25% black soldier fly larvae flour and 20% Moringa leaves.

Table 6: Effects on profitability and sustainability

Characteristics	Diets					p
	T0	T1	T2	T3	T4	
FCE	1.01±0.29ᵇ	0.33±0.04ᵃ	0.35±0.05ᵃ	0.91±0.10ᵇ	1.06±0.18ᵇ	0.00
Price of a kg of feed (Euro)	0.55	0.39	0.43	0.44	0.45	0.00
ECR	0.56±0.16ᶜ	0.13±0.01ᵃ	0.13±0.01ᵃᵇ	0.41±0.04ᵇᶜ	0.48±0.08ᵈ	0.00
EPI	0.07	0.48	0.39	0.33	0.34	0.00
Profitability (Euro)	3.29±0.16ᵇ	3.72±0.02ᵈ	3.70±0.02ᶜᵈ	3.44±0.04ᵇᶜ	3.37±0.08ᵇ	0.00
VFMU (g/kg weight gain)	285.60ᵃ	310.00ᵃ	288.00ᵃ	948.00ᶜ	1 308.00ᵈ	0.00
FIFO	4.76ᵈ	3.45ᶜ	1.05ᵃ	1.13ᵇ	1.04ᵃ	0.00

a, b, c, and d: The averages bearing the same letters in the same line are not significantly different (P>0.05). P=Probability, ECR: Economic Conversion Rate EPI: Economic Profitability Index VFMU: Value of fish meal utilization FCE: Feed Conversion Efficiency T0: control diet formulated locally without black soldier fly larvae meal or Moringa leaves; T1: Diet containing 6.25% black soldier fly larvae meal and 5% Moringa leaves; T2: Diet containing 12.5% black soldier fly larvae meal and 10% Moringa leaves; T3: Diet containing 18.75% black soldier fly larvae meal and 15% Moringa leaves; T4: Diet containing 25% black soldier fly larvae meal and 20% Moringa leaves.

on growth (flavonoids, tannins, and terpenes) as reported by Kana *et al*. (2018). Finally, the high fat content due to black soldier fly larvae in this diet would have been favorable for fish weight gain (Gutierrez *et al*., 2004). Beyond an incorporation rate of 18.75% of BSFLM and 15% of Moringa leaves (T4), a decrease in weight gain was recorded. This could be due to the combination of anti-nutritional factors such as tannins, oxalates, and saponins contained in Moringa (Richter *et al*., 2003), and the high lipid content (22 to 28%) and chitin content (5.7 to 6.7%) of black soldier fly larvae which inhibit animal growth. In the T0 control diet, the low weight gain could be explained by the fact that local ingredients available in the Cameroonian market are generally of questionable quality, especially fish meal (Cameroon Tribune, 2005).

The specific growth rate (SGR) is generally referred to the daily weight increase in percentage of an animal. According to Brown (1997), it is used in aquaculture to determine the weight gained by the fish per day, as a percentage of its live weight. In the present study, the specific growth rate was significantly influenced by the BSFLM and MOL in the feed. In fact, fish fed with T3 ration recorded a significantly high (P<0.05) SGR as compared to those obtained from other treatment. As indicated for live body weight, the highest SGR can be attributed to the combination of good feed physical state (buoyancy), the active compound in BSFL and secondary metabolite in MOL (Aisyah *et al.,* 2022; Kamble *et al*., 2024).

The condition factor K reflects the plumpness and physiological status of the fish. It is an important index for the characterization of the nutritional and physiological states of fish. In this study, it was comparable among all the treatment diets, but greater than or equal to 1 with the fish in the T0, T2, and T3 treatments and less than 1 with the subjects in the T1 and T4 diets. According to Fulton (1902), treatments that recorded a condition factor K greater than or equal to 1 indicate a good physiological state of the animal during the trial period. Therefore, all the treatment offered proper conditions for fish growth.

No differences were observed in the stomatal indices of the fish regardless of the treatment considered. These results are consistent with those obtained by Jozefiak *et al*. (2019) who studied the effect of insect-based diets on the gastrointestinal health and growth performance of Siberian sturgeon. This could be due to the fact that the fish were in good condition and that the diets did not disrupt the physiological balance.

Many studies on the inclusion of black soldier fly larvae meal and Moringa leaves in fish feed have been published; however, very few address their effect on the organoleptic quality of the flesh. The sensory analysis results of the present study support that black soldier fly larvae meal and MOL can be used as feed ingredient in *Clarias gariepinus* without side effect on flesh taste.

The price per kilogram of feed was significantly higher ($P<0.05$) with the T0 diet (0.55 €), and lower in the local diets containing black soldier fly larvae meal and *Moringa oleifera* leaves (0.39 €, 0.43 €, 0.44 €, and 0.45 €) for T1, T2, T3, and T4 respectively. Proportionally, this variation represents a reduction of 18% to 29% in the price per kilogram of feed respectively for T4 and T1. in the same vein, the profitability of the system was lower with the control diet T0 (2.31± 0.31€) and higher in the treatments containing black soldier fly larvae meal and Moringa leaves (3.72 ± 0.014€; 3.70 ± 0.02€; 3.44 ± 0.04€; and 3.37 ± 0.08€) for T1, T2, T3, and T4 respectively. This represents an increase in profitability ranging from 45.91 to 60.84% by incorporating black soldier fly larvae meal and Moringa leaves in the diet. These results are similar to those obtained by Rawski *et al.* (2021), who studied the economic sustainability and profitability of aquaculture using black soldier fly larvae in diets for Siberian sturgeon. They achieved a decrease of 1.52€ in production costs by incorporating 5% of black soldier fly larvae in the feed. This could be justified by the high cost of fish meal and soybean meal on the market and the relatively low price of black soldier fly larvae and Moringa leaves. The high profitability recorded with the MOL and BSFLM indicated that this could be an effective strategy for promoting aquatic foods and increasing the uptake of this activity as profitability is a high decision-making factor in his adoption.

The incorporation of these ingredients leads to the improvement of sustainability of fish farming with FIFO ratio near to 1 for T2 and T4 diets. It can be therefore said that the incorporation MOL and BSFLM reduce the environmental footprint of fish farming.

Conclusion

The inclusion of BSFLM and MOL in the diets increases the buoyancy duration of the feed. The incorporation of 18.75% BSFLM and 15% MOL (treatment T3) recorded the highest growth performance of *Clarias gariepinus*. Moreover, this same treatment (T3) reduced the cost per kilogram of feed by 29%, thus increasing the profitability to 60.84% as compared to the local diet containing conventional ingredients. The fish flesh was accepted, and its taste was not altered by the black soldier fly larvae meal and Moringa leaves. Therefore, treatment T3 containing 18.75% BSFLM and 15% Moringa leaves can be used for ecological and economical production of African catfish without altering its taste and acceptability by consumers. Moreover, this research highlights the environmental, economic value of local feed resources like moringa leaves and black soldier flies' larvae for the promotion of aquaculture in developing countries. Therefore, further research can evaluate explore different combinations of BSFLM and MOL on other fish species.

Ethical Statement

Authors strictly fellow up rule and regulation regarding animal as edited by the university of Dschang protocol.

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Author Contribution

M.K.H., designed the study, designed the data collection and analysis, interpreted the results, and review the manuscript. T.L., generated the research idea, participated in the design and conducted the study, data collection, analysis, and write the manuscript. T.P; D.Z; M.J; K.J.R and E.T participated in the design of the study and reading of the manuscript

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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