



Assessment of *Cirina butyrospermi* Caterpillar Meal as a Sustainable Protein Source for Aquarium-Reared Brazilian Strain Tilapia (*Oreochromis niloticus*) Larvae

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ABSTRACT

In an effort to identify sustainable alternative protein sources for larval tilapia and reduce dependence on conventional animal-based proteins in aquaculture, a study was conducted at the Nangui Abrogoua University fish farm. The experiment utilized *Oreochromis niloticus* (Brazil strain) and aimed to evaluate fry growth and the nutritional impact of incorporating shea caterpillar (*Cirina butyrospermi*) meal into the diet. This approach aligns with sustainability goals by minimizing reliance on fishmeal. Fry with an initial weight of $0.035 \pm 0.01\text{g}$ were fed three experimental diets containing 15% (A15), 20% (A20), and 25% (A25) *C. butyrospermi* meal. These diets were formulated using locally sourced agricultural by-products and compared to a control diet (AT), which did not include shea caterpillar meal. The base composition of the formulated diets included soybean meal, cottonseed meal, rice bran, wheat bran, and vegetable oil. The experiment was carried out in twelve aquaria, with three replicates per dietary treatment, and a stocking density of 4.13 fry/L, equivalent to 60 fry per aquarium. After 60 days of rearing, the feed conversion ratios (FCR) were recorded as 1.87 ± 0.34 (A15), 1.43 ± 0.34 (A20), and 1.97 ± 0.44 (A25). In contrast, the control diet (AT) exhibited a significantly higher FCR of 2.79 ± 0.51 . By the end of the trial, survival rates were equal to or exceeded 93.75% across all dietary treatments.

INTRODUCTION

The global demand for food—particularly animal-based proteins—continues to rise due to population growth and the expansion of middle-income classes (Fages & Bricas, 2017). In 2001, the global population was approximately 6.2 billion, and this figure is projected to surpass 11 billion by the end of the century, a trend expected to drive up food prices and place increasing pressure on the livestock production sector (FAO, 2013a). In its report, *The Shadow of Livestock Farming*, the FAO (2009) projected that global demand for livestock products would double over the next 50 years—from 229 million tonnes to 465

million tonnes. Meeting this demand requires intensification of conventional feed production, including increased reliance on fishmeal.

By 2016, global animal feed production had surpassed one billion tonnes (FAO, 2013b). Fishmeal remains a primary protein and lipid source in livestock feed. According to Amara (2010), the livestock sector captures and processes large quantities of fish to meet nutritional needs. At the same time, global fish consumption is increasing at a rate of approximately 1.5kg per capita per year, exacerbating pressure on fish stocks and contributing to supply shortages (Naylor *et al.*, 2000).

The excessive exploitation of wild fish stocks for fishmeal and fish oil not only disrupts aquatic food webs but also threatens the long-term sustainability of these marine resources. Fishmeal supply is inherently unstable, as it depends on wild-caught forage fish whose availability cannot be guaranteed. In the pursuit of ecologically sustainable alternatives, numerous studies have investigated the use of insects as viable protein sources for animal feeds (Van Huis, 2003; Kelemu *et al.*, 2015). Insects present a promising alternative due to their high protein content (ranging from 40% to 75% of dry weight), balanced amino acid profiles, and variable lipid levels (Verkerk *et al.*, 2007).

One notable candidate is *Cirina butyrospermi*, a caterpillar species that feeds on *Vitellaria paradoxa* (the shea butter tree). Its larvae contain approximately 63% protein (Ouedraogo, 1993) and show promise as a dietary component for carnivorous fish species, including *Clarias gariepinus* (Anvo, 2016). Anvo further suggests that caterpillar meal may effectively replace conventional protein sources in the diets of larval *C. gariepinus*, potentially reducing production costs.

Given the ongoing challenges faced by fish breeders in sourcing affordable, sustainable feed ingredients, increasing the use of insect-derived proteins is essential for reducing dependence on marine fish resources—currently the primary source of fishmeal. In light of these considerations, this study aimed to evaluate the feasibility of incorporating shea caterpillar meal (*Cirina butyrospermi*) into the diet of *Oreochromis niloticus* (Brazil strain) larvae, using locally available agricultural by-products. Specifically, the research investigates the effects of experimental diets containing varying levels of shea caterpillar meal on feed conversion efficiency and larval growth performance, with the goal of determining optimal inclusion levels for maximizing growth in this species.

MATERIALS AND METHODS

Research site overview

The trials were conducted at the fish farm of Nangui Abrogoua University in Côte d'Ivoire. The farm is located within the university campus (5°23'30" N, 4°0'56" W) in Abidjan, the country's economic capital. The university lies along the expressway connecting the Abobo and Adjamé districts, with a spinning mill forming the boundary between the institution and Banco National Park.

Breeding structures and experimental fish

The experimental setup comprised 12 aquaria, with three aquaria assigned to each dietary treatment. Each aquarium held a usable water volume of 14.5 liters, with dimensions of 32.4cm × 21.4cm × 21cm. The experimental fish consisted of *Oreochromis niloticus* (Brazil strain) larvae, with an average initial body weight of 0.035 ± 0.01 g. The larvae were obtained from broodstock reared under controlled conditions, where females and males averaged 100 and 200g, respectively. A sex ratio of 3:1 (three females per male) was maintained during breeding.

Experimental diets and diet preparation

Four isoproteic experimental diets were formulated using plant-based raw materials, with varying inclusion levels of shea caterpillar (*Cirina butyrospermi*) meal. The raw ingredients used included rice bran, wheat bran, soybean meal, cottonseed cake, vegetable oil, sodium chloride (salt), shell flour, and a vitamin-mineral premix (VMP). The diets were designated as AT, AC15, AC20, and AC25, containing 0%, 15%, 20%, and 25% shea caterpillar meal, respectively (Table 1). The proximate composition of the diets (expressed as a percentage of dry matter) is presented in Table (2).

The control diet (AT) contained only plant-derived protein sources, while the AC15, AC20, and AC25 diets incorporated increasing amounts of *C. butyrospermi* meal. All diets were prepared in powdered form.

Prior to diet formulation, *C. butyrospermi* caterpillars were manually harvested from shea trees in the PORO region (Korhogo), thoroughly washed, and pre-cooked at 100°C. They were then oven-dried at 65°C for 72 hours. The dried larvae were finely ground and stored for subsequent analysis and incorporation into feed formulations.

For diet preparation, all raw ingredients were first ground to a particle size of 1 mm using a locally fabricated hammer mill, followed by sieving through a 500-micron mesh mosquito net. Each ingredient was then individually sieved, weighed, and manually mixed according to the designated formulation until a homogeneous powdered blend was achieved. Vegetable oil, salt, and the vitamin-mineral premix were subsequently added and thoroughly homogenized by hand to ensure even distribution. The final diets were stored in airtight containers to preserve freshness and nutrient stability.

Table 1. Composition and proportions of ingredients (g/100 g) in diets (AC15, AC20, AC25 and AT)

Ingredient (%)	Food processing			
	Feed AC15	Feed AC20	Feed AC25	Feed AT
Soybean meal	31	27	22	32
Rice bran (low-flour)	13	15	16	20
Wheat bran	14	15	17	20
Cotton cake	22	18	15	23
<i>Cirina butyrospermi</i> larvae meal	15	20	25	00
Salt	1.5	1.5	1.5	1.5
Vegetable oil	1.5	1.5	1.5	1.5
CMV*	0.5	0.5	0.5	0.5
Shell	1.5	1.5	1.5	1.5
Total	100	100	100	100

Table 2. Chemical composition (% dry matter) of experimental diets

Settings	AC15	AC20	AC25	AT
Dry matter	73.48	70.48	67. 56	82.96
Protein	35.33	35.36	35. 38	28.90
Lipids	9.38	9.54	9.78	9.13
Center (ASH)	5.13	5.04	4.93	5.28
Fiber	7.46	7.02	6.64	8.35

Experimental design and procedures

Prior to the start of the experiment, *Oreochromis niloticus* larvae were batch-counted in groups of 20, weighed, and randomly distributed across 12 aquaria. Each aquarium was stocked with 60 larvae, resulting in a total of 720 individuals divided into four dietary treatments, each with three replicates.

The experimental diets were administered over a 60-day period. Fish were fed ad libitum at a daily ration rate of 0.025g per larva. From day 1 to day 30 (D01–D30), the daily ration corresponded to 1.5g per batch, after which feeding was adjusted to 10% of the

total live weight until the end of the experiment. Feeding occurred hourly from 09:00 to 16:00 to optimize nutrient uptake and ensure consistent consumption.

Growth assessment

Growth performance was assessed at 16-day intervals. Measurements were conducted at 06:00 following every 15 days of feeding. During each evaluation, 30 larvae were randomly sampled and weighed in groups of five using a CONTANT electronic scale (precision: 0.1 g; maximum capacity: 2000 g). At the conclusion of the 60-day trial, 30 fries were randomly selected from each aquarium for individual weight measurements to facilitate statistical analysis. Various zootechnical performance parameters were then calculated based on the recorded data. Additionally, all aquaria were emptied at the end of the trial to assess survival rates.

Water quality management

To maintain optimal water conditions, the self-contained aquaria were filled with dechlorinated tap water and continuously aerated using bubblers. A filtration system was employed to ensure water quality, and temperature was thermoregulated at 27°C. All tanks were conditioned at least 48 hours prior to stocking and consistently maintained throughout the experiment.

Water quality parameters—including pH, dissolved oxygen, and temperature—were measured daily at two intervals: between 06:00 and 07:00, and again between 13:00 and 14:00. Measurements were conducted *in situ* using calibrated digital probes. The sensors were immersed in the water, and real-time data were recorded upon activation of the appropriate device functions.

Assessment of zootechnical performance and feed utilization efficiency

Throughout the 60-day rearing period, key zootechnical parameters were evaluated, including:

- Specific growth rate (SGR)
- Weight gain (WG)
- Survival rate (SR)
- Feed conversion ratio (FCR)

These indicators provided essential insights into the growth dynamics and feeding efficiency of the experimental fish and were calculated using standard aquaculture performance formulae.

- Weight gain (GM. g) = Final weight (g) - initial weight (g)
- Daily weight gain (DWG. g/d) = (Final weight (g) - initial weight (g)) / feeding time
- Specific growth rate (SGR. %/day) = $100 \times [\ln(\text{final weight}) - \ln(\text{initial weight})] / \text{feeding time}$
- Protein efficiency ratio (PER) = Fresh weight gain / protein intake
- Fed conversion ratio (FCR) = Quantity of dry feed distributed / fresh weight gain

Statistical analysis

The collected data on zootechnical performance parameters—including weight gain (WG), daily weight gain (DWG), protein efficiency ratio (PER), feed conversion ratio (FCR), survival rate (SR), and water quality—were subjected to one-way analysis of variance (ANOVA) using R software (version 4.1.3). The ANOVA was conducted to evaluate differences between the control and experimental dietary groups. Following the ANOVA, the Kruskal–Wallis test was applied for multiple comparisons to determine statistically significant differences among the groups. A significance level of 5% ($P < 0.05$) was used for all statistical analyses (Al Sulivany *et al.*, 2024b).

RESULTS

1. Physico-chemical parameters of farm water

The mean values of the physico-chemical water parameters recorded during this experiment are presented in Table (3).

These parameters were under control and no variations were observed.

Table 3. Mean values of physico-chemical parameters in the rearing environment

		Dietary treatments			
Settings		A15%	A20%	A25%	AT 0%
Temperature (°C)		27.16 ± 0.8 ^a	27.18 ± 0.2 ^a	27.16 ± 0.12 ^a	27.15 ± 0.12 ^a
Dissolved oxygen (mg/L)		4.4 ± 0.28 ^a	4.5 ± 0.04 ^a	4.5 ± 0.14 ^a	4.4 ± 0.26 ^a
pH		7.87 ± 0.09 ^a	7.88 ± 0.06 ^a	7.86 ± 0.07 ^a	7.79 ± 0.10 ^a
TDS (ppm)		163.05 ± 0.39 ^a	163.08 ± 0.36 ^a	163.06 ± 0.38 ^a	162.08 ± 0.35 ^a
COND (µS/cm)		367.50 ± 7.62 ^a	365.60 ± 27.90 ^a	364.53 ± 0.86 ^a	365.68 ± 20.34 ^a

In each line, values with the same subscript letter are not significantly different ($P > 0.05$). Results are expressed as: Mean ± SD (standard deviation) from two replicates. in each line. values (Means ± ECT) marked with different letters are significantly different ($P < 0.05$). whereas values sharing at least one letter are not significantly different ($P > 0.05$).

- AC15: practical diet containing 15% of *Cirina butyrospermi* larvae meal
- AC20: practical diet containing 20% of *Cirina butyrospermi* larvae meal
- AC25: practical diet containing 25% of *Cirina butyrospermi* larvae meal
- AT: practical diet containing 0% of *Cirina butyrospermi* larvae meal

2. Weight growth

Fig. (1) illustrates the weight gain dynamics of *Oreochromis niloticus* over the 60-day experimental period under four dietary treatments: AC15, AC20, AC25, and AT. The observed growth trends among the fry allowed for classification into four distinct performance groups. Fish fed the AC20 diet exhibited the highest weight gain, followed by those fed the AC15 diet. The third group, which received the AC25 diet, showed a noticeably different growth pattern compared to the fourth group, which was fed the control diet (AT). The fry in the AT group demonstrated the lowest growth performance by the end of the experiment.

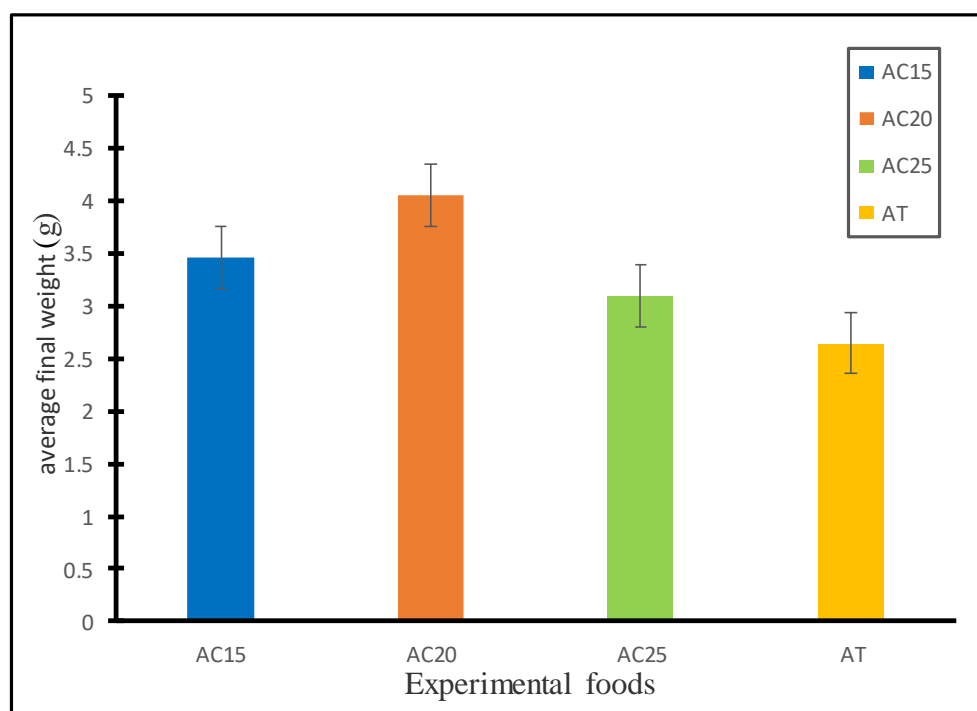


Fig. 1. Variation in the final mean weight of *Oreochromis niloticus* larvae, brazilian strain, under different feeding treatments in aquarium conditions

- AC15: Local practical diet food containing 15% of *Cirina butyrospermi* larvae meal
- AC20: practical diet Local food containing 20% of *Cirina butyrospermi* larvae meal;
- AC25: practical diet Local food containing 25% of *Cirina butyrospermi* larvae meal
- AT: practical diet containing 0% of *Cirina butyrospermi* larvae meal

3. Growth performance and feed utilization

The evaluation of growth performance over the 60-day feeding trial—measured by final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), survival rate, and protein efficiency ratio (PER)—is summarized in Table (4). By the

end of the trial, survival rates exceeded 90% across all dietary treatments, ranging from 91.66% (AT) to 96.11% (AC20). A statistically significant difference ($P < 0.05$) was observed in survival rates among fish subjected to the different dietary treatments.

With respect to growth parameters, a significant difference ($P < 0.05$) was found in FBW, SGR, and daily weight gain (DWG) among fish receiving the experimental diets. Fish fed the AC20 diet exhibited the best growth performance, recording the highest final body weight (4.84 ± 0.25 g) and specific growth rate ($8.74 \pm 0.45\%$ per day). In contrast, the lowest FBW (2.63 ± 0.57 g) and SGR ($7.68 \pm 0.35\%$ per day) were observed in fish fed the control diet (AT).

The feed conversion ratio fluctuated between 1.43 and 2.79. with the most efficient FCR (1.43) obtained in the A20 dietary group. whereas the least effective conversion (2.79) was associated with the control AT diet. Similarly. the AC20-fed group exhibited the highest PER (2.09 ± 0.54). surpassing the values reported for alternative dietary treatments

Table 4. Zootechnical parameters of *Oreochromis. niloticus* fed on four diets

Setting	Dietary treatments			
	AC15	AC20	AC25	AT
Initial weight : (g)	0.035 ± 0.001^a	0.035 ± 0.001^a	0.035 ± 0.001^a	0.035 ± 0.001^a
Final weight : Pmf (g)	3.62 ± 0.67^b	4.84 ± 0.25^c	3.35 ± 0.72^b	2.63 ± 0.57^a
Weight gain : (g)	3.58 ± 0.67^b	4.80 ± 0.25^c	3.31 ± 0.72^b	2.60 ± 0.57^a
Daily weight gain: (g/ d)	0.06 ± 0.01^b	0.08 ± 0.02^c	0.05 ± 0.01^b	0.04 ± 0.01^a
Specific growth rate (%/ day)	8.25 ± 0.33^a	8.74 ± 0.45^a	8.10 ± 0.39^a	7.68 ± 0.35^a
Feed conversion ratio (FCR)	1.87 ± 0.34^b	1.43 ± 0.34^a	1.97 ± 0.44^b	2.79 ± 0.51^c
Protein Efficiency ratio (PER)	1.56 ± 0.29^b	2.09 ± 0.54^c	1.50 ± 0.33^{ab}	1.28 ± 0.28^a
Survival rate : Ts (%)	94.44 ± 0.66^b	96.11 ± 0.60^d	93.88 ± 0.33^c	91.66 ± 0.66^a

In each row, values with the same subscript letter are not significantly different ($P > 0.05$). Results are expressed as Mean \pm SD (standard deviation) based on two replicates. Values marked with different letters are significantly different ($P < 0.05$), while those sharing at least one letter are not significantly different ($P > 0.05$).

- AC15: Practical diet containing 15% *Cirina butyrospermi* larvae meal
- AC20: Practical diet containing 20% *Cirina butyrospermi* larvae meal
- AC25: Practical diet containing 25% *Cirina butyrospermi* larvae meal
- AT: Control diet containing 0% *Cirina butyrospermi* larvae meal

DISCUSSION

The physico-chemical parameters recorded during the experiment remained within the optimal ranges recommended for *Oreochromis niloticus* farming in tropical environments, particularly for temperature (Ndour *et al.*, 2011; Sarr *et al.*, 2013; Faye *et al.*, 2018; Chikou *et al.*, 2019). These conditions did not negatively affect larval growth. Observations made during the controlled trials showed minimal fry mortality, and the high survival rates further support the conclusion that water quality was well-suited to tilapia fry development (Abdel-Tawwab *et al.*, 2010).

These results also reinforce the nutritional value and quality of the tested feed formulations, confirming their adequacy for supporting fry growth. Mortality incidents generally occurred following sampling operations and were likely due to physical stress, such as handling injuries or water current disturbances. This indicates that observed mortality was primarily caused by external handling stress rather than dietary or environmental factors.

Survival rates in this study were satisfactory when compared to the 80% reported by Mohapatra and Patra (2013), who found that *Lemna minor* at 15% inclusion could partially replace fish protein in *Cyprinus carpio* fry diets, and to the findings of Medard *et al.* (2018), who reported survival rates below 90% in *O. niloticus* fry fed diets containing earthworm and maggot meal. However, the survival rates observed in the present study were slightly lower than those reported by Ly and Ba (2015) (99.36–100%) using soy flour-based diets and Dibala *et al.* (2018), who achieved 100% survival in *O. niloticus* juveniles fed plant protein diets in Burkina Faso.

Growth performance indicators—such as weight gain (WG), daily weight gain (DWG), feed conversion ratio (FCR), and protein efficiency ratio (PER)—differed significantly ($P < 0.05$) among all dietary treatment groups when compared to the control diet (AT). Notably, DWG values in fish fed shea caterpillar-based diets were higher than those in the control group: AC15 (0.06 g/day), AC20 (0.08 g/day), AC25 (0.05 g/day), and AT (0.04 g/day). These values are consistent with those reported by Fiogbé (2004) and Silue *et al.* (2024) (0.02–0.05 g/day) in similar rearing systems, but considerably lower than the 0.71–1.11 g/day observed in ponds by Houndonougbo *et al.* (2017) in Benin. Such differences in growth rates are likely due to variations in rearing environments; fish in ponds benefit from natural feed sources, whereas those in this study received exclusively formulated diets in a controlled aquarium setting.

Specific growth rates (SGR) recorded were as follows: AC15 ($8.74 \pm 0.45\%$ /day), AC20 ($8.25 \pm 0.33\%$ /day), AC25 ($8.10 \pm 0.39\%$ /day), and AT ($7.68 \pm 0.35\%$ /day). Feed conversion ratios (FCR) were: AC15 (1.87 ± 0.30), AC20 (1.43 ± 0.34), AC25 (1.97 ± 0.44), and AT (2.79 ± 0.51). Protein efficiency ratios (PER) were: AC15 (1.56 ± 0.29),

AC20 (2.09 ± 0.54), AC25 (1.50 ± 0.33), and AT (1.28 ± 0.28). The FCR values in this study were higher than those reported by **Bamba (2007)** (1.1–1.3), possibly due to differences in rearing systems—previous studies were conducted in concrete tanks exposed to ambient conditions, allowing access to naturally occurring feed sources.

Overall, the AC20 diet yielded the best zootechnical performance among all treatments. Among the three experimental diets, the highest growth and feed utilization efficiency were recorded with AC20, followed by AC15. These findings highlight the impact of shea caterpillar meal inclusion levels on tilapia growth. The improved performance of fish fed diets containing *Cirina butyrospermi* meal may be attributed to the higher protein content (~35%) and optimized nutritional composition of the experimental feeds.

Previous research has shown that dietary fiber levels exceeding 10% can reduce digestive enzyme efficiency and thus impair feeding performance (**Francis *et al.*, 2001; Krogdahl *et al.*, 2010**). Similar observations were made by **Capuano (2017)**, who reported reduced growth in *O. niloticus* fed diets containing more than 10% fiber. The NRC also recommends limiting fiber content in tilapia diets to below 8% to avoid growth reduction. The improved growth seen in this study suggests that the experimental diets met nutritional requirements effectively by offering a high protein content and moderate fiber levels.

Based on these findings, the protein content and inclusion of *Cirina butyrospermi* meal appear to be key factors contributing to the observed differences in growth performance among the dietary treatments.

CONCLUSION

This study demonstrates that the inclusion of shea caterpillar (*Cirina butyrospermi*) meal in tilapia fry diets significantly enhances zootechnical performance compared to the control diet based solely on vegetable proteins. Among the tested formulations, the diet containing 20% shea caterpillar meal (AC20) yielded the best results in terms of growth rate and feed conversion efficiency. The findings highlight the potential of shea caterpillar meal as a viable alternative protein source in tilapia fry nutrition, offering a sustainable and locally available solution to reduce reliance on conventional feed ingredients such as fishmeal. Incorporating 20% shea caterpillar meal provides a nutritionally balanced approach that supports optimal growth under controlled rearing conditions. These results open new avenues for the use of insect-based proteins in sustainable aquaculture and contribute to the diversification of feed ingredients, which is essential for enhancing productivity and environmental resilience in the sector.

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