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Evaluation of palm weevil larvae meal (*Rhynchophorus phoenicis*) as an alternative protein source for pre-pregnancy juvenile tilapia (*Oreochromis niloticus*) (LINNE, 1758) strain "Brazil" in aquaria

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Abstract
With the aim of promoting the feeding of tilapia for good production in aquaculture farms in Côte d'Ivoire, three diets with incorporation rates of 15%, 20% and 25% of *Rhynchophorus phoenicis* weevil larvae meal were prepared using a feed based on agricultural by-products as a control diet (AT). A total of 360 fish, with an average weight of 1.51±0.05 g, were placed in twelve aquaria, with 30 fry per aquarium in triplicate. The fry were fed daily ad libitum at a ration rate of 10% of their live weight for the first two weeks and 5% for the rest of the rearing period. After 90 days of rearing, the best feed conversion was obtained with the R_{25} diet (1.33±0.17), followed by the R_{20} diet (1.38±0.22; 20.42±0.85 g). The lowest daily growth (0.13±0.01 g/day) and the highest conversion index (1.94±0.23) were recorded with the control. Survival rates were greater than or equal to 92.85%. At the end of this study, weevil larvae meal can be considered as à good alternative raw material for tilapia feed.

Keywords: *Oreochromis niloticus*, feeding, fry, *Rhynchophorus phoenicis*, growth

1. Introduction
The fisheries and aquaculture sector is the mainstay of fisheries production in Côte d'Ivoire. The contribution of aquaculture to fisheries production remains insignificant, even though the country has considerable natural assets (à fairly flat landscape and a large hydrographic network), (Failler *et al*., 2019) [8]. In addition, the country has 150,000 ha of lagoons, 350,000 ha of lakes and numerous lowlands. In fact, national fisheries production (fishing and aquaculture) contributes only 10% (including 4% for aquaculture) to meeting the needs of the Ivorian population (MIRAH). According to MIRAH, annual fish production in Côte d'Ivoire is between 70,000 and 100,000 tonnes, while overall requirements are estimated at 652,864 tonnes in 2019, making the country dependent on massive imports of frozen fish, despite its considerable potential in terms of natural resources and human skills. This shows that supply is lower than national demand. In order to achieve food security for the population, the State has initiated a project called the National Aquaculture Development Strategy, which aims to make aquaculture a successful and prosperous sector. However, the development of this activity is hampered by a lack of high-performance feed on the local market at prices that fish farmers can afford. Fish feed accounts for 60-70% of production costs (FAO, 2015) [9]. According to Coulibaly *et al* (2021) [5], feed is the biggest cost item in the production cycle of commercial fish. Fishmeal is one of the main components of fish feed in aquaculture, which creates competition with humans, just as demand for aquaculture feed increases fishing pressure on wild fish resources (Médale *et al*. 2013) [13]. The use of this imported raw material is thought to be at the root of this cost, which aquaculturists consider to be high. To overcome this problem, a great deal of work has been done to identify local protein sources that can replace fishmeal and fish oil (Akegbejo *et al*. 2008) [3]. Among these sources, plant proteins have been identified and used successfully in fish farming (Azaza *et al*. 2006) [4].

One of the constraints identified for the full use of these plant proteins is the presence of anti-nutritional factors. In the search for new sources of ecologically sustainable feed, numerous studies have shown the alternative role that insects could play in animal feed. Replacing feed based on fishmeal and proteins of plant origin with proteins of animal origin other than fishmeal could reduce production costs and improve farm profitability. The aim of this study is to contribute to the development of feeds based on palm weevil larvae meal combined with local agricultural by-products, accessible to fish farmers in order to improve the profitability of aquaculture operations.

2.2 Rearing facilities and experimental fish

The experimental set-up consists of 12 aquariums, three for each food treatment. Each aquarium has a usable water volume of 14.5 litres (Length x Width x Height: 32.4 cm x 21.4 cm x 21 cm). Each of the aquariums used is equipped with a bubbler acting as an aerator. The fish used for the tests were tilapia *Oreochromis niloticus* "Brazil strain" fry with a mean initial weight of 1.51±0.05 g. These fry were produced from broodstock with an average body weight of 180±0.96 g for the females and 250±0.87 g for the males using a sex ratio of 3:1, i.e. three females to one male.

2.3 Experimental feed

During the trials, four (4) diets were formulated based on vegetable raw materials with the addition of weevil larvae meal. The raw materials used to formulate the diets consisted of low-grade rice flour, wheat bran, various cakes (soya and cotton), vegetable oil, cooking salt, shell meal and a mineral-vitamin complex (MVC). These formulated diets, named AT, A_{15}, A_{20} and A_{25}, differ in their composition, with 0, 15, 20 and 25% larval meal respectively.

Table 1: Composition and incorporation proportions of the ingredients (g/100g) of the diets (AT, A_{15}, A_{20} and A_{25})

	Food treatment			
Ingredients (%)	**AT**	**Feed A_{15}**	**Feed A_{20}**	**Feed A_{25}**
Soya meal	32	33	26	22
Rice bran (low-flour)	20	14	15	16
Wheat bran	20	14	17	17
Cotton cake	23	19	17	15
White palm maggot flour	00	15	20	25
Salt	1.5	1,5	1,5	1,5
vegetable oil	1	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

2.4-Preparation of weevil larvae meal

Rhynchophorus phoenicis were used to produce weevil larvae on a substrate. After hatching, the weevil larvae were fed until they reached the stage of larval development. They were then harvested, placed in jars to be cleaned, sacrificed in boiling water, wrung out, sorted, distributed on trays and transferred to the laboratory to be dried in an oven at 70°C. After drying, larva meal was obtained by grinding the dried larvae and then grinding them into meal for use in formulating test feeds. To manufacture the feeds, the raw ingredients were ground (1 mm in diameter) using a locally manufactured hammer mill and passed through a sieve made from 500 micron mesh mosquito netting. The ingredients of each feed were sieved, weighed and mixed according to the feed formula until a homogeneous powder was obtained. The manufactured feeds were packaged in jars.

2.5 Breeding management and monitoring

The experiments consisted of feeding the experimental feed to tilapia *Oreochromis niloticus* fry, with an average weight of 1.51±0.05 g, for 90 days. To load the aquaria, 10 fry were weighed. Several weighings were carried out to reach the stocking density. The stocking density in the experimental aquaria was 30 fry per aquarium, i.e. a total of 360 fry for the twelve (12) aquaria. The fish were randomly distributed in twelve aquariums, thus forming four triplicate treatments. The fry were fed daily ad libitum at a ration rate of 10% of their live weight for the first two weeks and 5% for the remainder of the rearing period. The daily food rations were served four times a day (9am, 12pm, 2pm and 4pm). Control fishing was carried out every 15 days. A sample of 30 fish was caught and weighed individually per aquarium to monitor their growth. This activity takes place every 15 days at 7am and the fish are immediately returned to the aquariums after weighing.

At the end of the 90th day of rearing, all the fry were sampled and their individual weights were measured for statistical comparison. Various zootechnical performance parameters were calculated from this data. In addition, at the end of the experiment, all the aquaria were emptied of their contents to assess the survival rate.

2.6 Water quality parameters

Monitoring the quality of the aquarium water involved taking readings of pH, dissolved oxygen and temperature *in situ* every two hours between 7am, 12pm and 5pm. The calibrated measuring instruments were switched on, then the various probes were immersed in the water and the desired function was selected in order to obtain the value of the parameter on the dial.

2.7 Détermination of zootechniques performance parameters

During the 90 days of rearing, the zootechnical growth parameters (specific growth rate, weight gain, survival rate) and the level of feed utilisation (consumption index) were calculated according to the formulae explained below.

- **Survival rate (SR):** This is the percentage of individuals having survived at a time (t) in the experiment in relation to the number of fish loaded.
- **Specific weight growth rate (SWGR):** Expressed as a percentage per day (%. D-1), it represents the daily weight growth rate.
- **Average weight gain (AWG):** This is the average weight gain over a given time interval.

- **Average daily gain (ADG):** This is the weight gain per unit of time. It represents daily weight growth.
- **Apparent consumption index (Ica):** This expresses the quantity of feed in kg required to produce 1 kg of net live weight of fish. It is calculated on the assumption that all the feed distributed has been consumed.
- **Protein Efficiency Coefficient (PEC):** The protein efficiency coefficient reflects the fish's capacity to use the proteins provided by the feed and convert them into body mass.

2.8 Statistical analysis

The statistical processing of the data for the various zootechnical parameters studied, i.e. weight gain (GP), daily weight gain (GPj), protein efficiency ratio (PER), feed conversion ratio (FC), survival rate (SR) and water quality, were subjected to an analysis of variance with a hierarchical classification criterion (ANOVA1) using R software version 4.1.3. The aim of the ANOVA was to determine whether or not there was a statistically significant difference ($p<0.05$) between the parameters resulting from the different feed treatments used. A significance level of 5% was used for these comparisons.

3. Results

3.1 Water quality

Data-t-on the physico-chemical parameters of the aquarium water are given in **Table II**. The water temperatures recorded during the rearing experiments in the different treatments ranged from 27.52±0.66 ° C to 27.65±0.67°C. Mean values ranged from 27.52±0.66°C to 27.54±0.60°C. Dissolved oxygen recorded was between 3 and 4 mg/L with mean values between 3.28±0.74 mg/L (AT) and 3.69±0.45 mg/L (A_{25}). As for pH, the average values recorded ranged from 7.00±0.17 (AT) to 7.28±0.27 (A_{15}). For the four treatments carried out, There was a significant difference ($p<0.05$) between the treatments in terms of aquarium water temperature, pH and dissolved oxygen.

Table 2: Average values for physico-chemical parameters of aquarium water.

	Food traitements			
Parameters	**A_{15}**	**A_{20}**	**A_{25}**	**AT**
O2	3,64±0,47^{ab}	3,67±0,45^{b}	3,69±0,45^{b}	3,28±0,74^{a}
PH	7,28±0,27^{b}	7,02±0,12^{a}	7,04±0,19^{a}	7,00±0,17^{a}
T°C	27,65±0,67^{a}	27,54±0,60^{a}	27,62±0,58^{a}	27,52±0,66^{a}

The results were expressed as the mean±standard deviation (SD) of three repetitions for each test food. On each line, the values (Mean±SD) On each line, the values with the same subscript letter are not significantly different (p>0.05).

3.2 Weight growth

The average weight growth of the fish over all the feeding trials (Figure 2) of *O. niloticus* "Brazil strain" fry fed on diets A_{15} ; A_{20} ; A_{25} and AT, showed almost similar growth after 15 days of fry feeding. After this period, three groups can be distinguished. Fish fed with feed (A_{20}) and feed (A_{25}) showed greater weight growth than the other fish. In ascending order of weighted growth, fish fed the AT feed, followed by fish fed the A_{15} feed and then fish fed the A_{20} and A_{25}

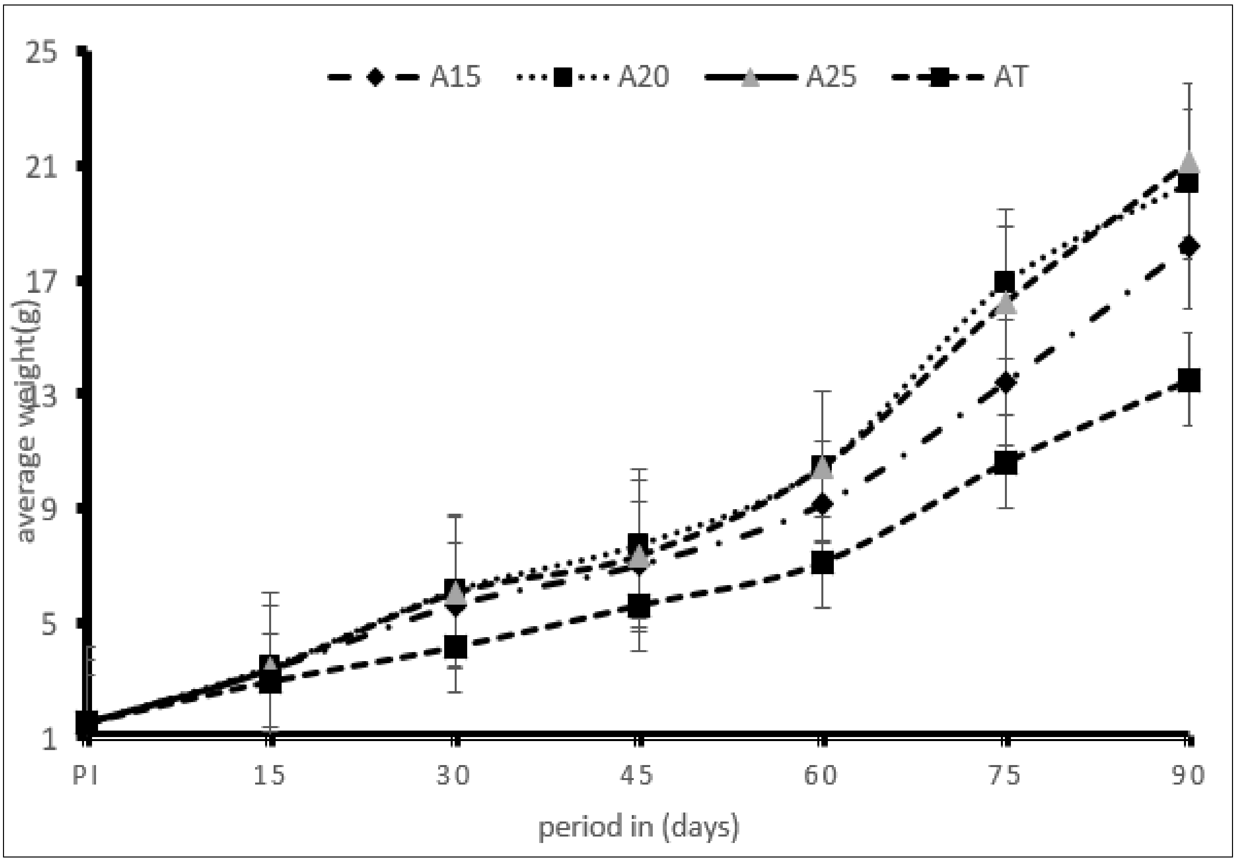

Fig 2: Average weight growth of fish as a function of test feed

3.3 Zootechnical parameters

The results of the growth parameters (average final weight, average daily weight gain, specific growth rate) and food processing (apparent consumption index, protein efficiency coefficient) as well as the survival rate in *Oreochromis niloticus* in the larval phase are recorded in Table III. The zootechnical parameters were also satisfactory with the incorporation of palm weevil larvae meal. These parameters show that the average final weights of the fish ranged from 13.51±1.25 g for the control feed and from 18.21±2.37 to 21.16±3.18 g for those fed the feed containing palm weevil larvae meal. The analysis showed that there was a significant difference (p<0.05) between the final weights of the treatments. The growth rates (TCS) calculated, varied between 2.53% in fish fed the AT feed, and from 2.88% to 3.05% in fish fed the feeds (A_{15},A_{20} and A_{25}), with a significant difference between treatments. At the end of the experiment, mean feed conversion rates were 1.33±0.17, 1.38±0.22, 1.40±0.18 and 1.94±0.23 for the A_{25},A_{20}, A_{15} and AT diets respectively. Fish fed diets containing palm weevil larvae meal (15, 20, 25%) showed good weight growth. This growth was higher in fish fed diets containing 20% and 25% of palm weevil larvae meal as protein, with Specific Growth Rates (SGR) of 3,00±0.19 (A_{20}), 3.05±0.16 $(A)_{25}$ and a Feed Conversion Rate (FCR) of 1.33±0.17 (A25), 1.38±0.22 (A_{20}) indicating good digestibility of the feed due to the nature of the ingredients. As for survival rates, analysis of variance shows no significant difference between these values. Average survival rates ranged from 93.33±0.62 (R_{25}) to 92.85±0.87 (AT).

Table 3*:* Zootechnical parameters *of Oreochromis. niloticus* fed with four *test* feeds *(A_{15}, A_{20}, A_{25} and AT)*

	Food trématent			
Parameters	**A_{15}**	**A_{20}**	**A_{25}**	**AT**
Initial weight: Pmi (g)	1,51±0,05^a	1,51±0,05^a	1,51±0,05^a	1,51±0,05^a
Final weight: Pmf (g)	18,21±0,37^b	20,42±0,85^c	21,16±0,18^c	13,50±0,25^a
Weight gain: GP (g)	16,70±0,37^b	18,91± 0,85^c	19,64±0,18^c	11,99±0,25^a
Daily weight gain: GPj (g/d)	0,19±0,02^b	0,21±0,04^c	0,22±0,03^c	0,13± 0,01^a
Consumption index (CI)	1,40±0,18^a	1,38±0,22^a	1,33±0,17^a	1,94±0,17^c
Specific growth rate: TCS (%/day)	2,88±0,14^b	3,00±0,19^c	3,05±0,16^c	2,53±0,11^a
Survival rate: Ts (%)	92,85±0,87^a	93,33±0,5^b	93,33±0,62^b	92,85±0,25^a

The results were expressed as : Mean±ECT (standard deviation) of three replicates. On each line, the values (Means±ECT) affected by different letters are significantly different (p<0.05) and those with at least one letter in common are not significantly different (P>0.05).A_{15} : Local food containing 15% insect meal; A_{20} : Local food containing 20% insect meal; A_{25} : Local food containing 25% insect meal; At: Local food not containing insect meal.

4. Discussion

The mean values of temperature, dissolved oxygen and PH observed in the aquaria remained within the recommended range for rearing *Nile* tilapia *Oreochromis niloticus* of 27-30 °C for good growth of this fish species. The temperature values (27.52 -27.65°C) recorded during this experiment are similar to those (24.1-28.5) reported by Sarr *et al.* (2013) [16] with intervals of (24 - 35°C). The zootechnical parameters of the individuals in all treatments were not affected by the physico-chemical parameters. The incorporation of palm weevil larvae meal into the fish feed did not appear to affect the survival rate of the fish. The survival rate of our results was similar to those of Coulibaly *et al* (2021) [5] who obtained an overall rate of 94% at the end of their work. The results of this experiment are lower than those of Ly and Ba (2015) [11] Who obtained a raté varying from 95 to 100% in juvenile *lates niloticus* fed with soya flour as well as those of Dibala *et al.* (2018) [6] who obtained 100% survival in tilapia (*Oreochromis niloticus*) fed for two months with vegetable protein in Burkina Faso. However, the mortalities recorded during the 90 days of rearing could be the result of manipulations during control fishing and, above all, the very small size of the fry, or either power cuts that cause the bubblers to stop working.

With regard to zootechnical performance, the average daily growth recorded in this experiment is higher than that of Abou *et al.* (2007) [2] in ponds (0.1 g.d-1) but lower than that of Houndonougbo *et al.* (2017) [10], who obtained values ranging from 0.71±0.13 to 1.11±0.13 in ponds in Cotonou (Benin) during 45 days of experiment. However, these daily weight gain values are similar to those of Fanda Ngandeu (2012), who reported a rate of 0.24 g/d. The specific growth rates (SGR) are higher than those of Dibala *et al* (2018) [6], who obtained a SGR ranging from 0.83 to 0.90%/d, but lower than those of Azaza *et al* (2006) [4], who recorded a value ranging from 5.11 to 5.97%/d. The feed conversion ratio obtained with the test feed over 90 days of rearing (1.33±0.1 7; 1.94±0.23) was lower than that of Coulibaly *et al.* (2021) [5], Who obtained higher feed conversion ratios, ranging from 2.8 to 8, and Dibala *et al.* (2018) [6], who obtained a value of 2.7.

5. Conclusion

This study assessed the effects of incorporating weevil larvae meal into the feed of *Oreochromis nilotuicus* fish for 90 days on the fry of this species. The zootechnical performance and nutritional values of this species were tested. Similarly, the quality of the fish rearing environment in relation to the incorporation of weevil larvae meal as a source of protein in the feed was evaluated during this experiment. The results of this study showed that weevil larvae meal has the potential to be incorporated into the feed of *Oreochromis niloticus* "Brazilian strain" fry. The results showed that a level of incorporation of 20% to 25% of weevil larvae meal improved growth performance. The performances observed for each of the rations studied show the potential use of this meal in *Oreochromis niloticus* 'brazil strain' fry.

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