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Valorization of Benthic Macroinvertebrates Produced from Pig Dung in *Clarias gariepinus* Fries Feeding

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Abstract

Fish feeding is one of the main factors hindering the development of fish farming in developing countries. The objective of this study was to determine the optimal artificial feed ration to be fed as a supplement to macroinvertebrate fed fry for semi-intensive production of *Clarias gariepinus*. To this end, the survival and growth performance of Clarias gariepinus fries fed with macroinvertebrates produced from pig dung and artificial feed were compared to those of fry fed with dry feed only. Fries with an initial weight of 0.52 ± 0.15 g were distributed in the 15 buckets with a density of 0.6 ind.L⁻¹ and grouped into four (04) treatments (T1, T2, T3 and T4) and a control (T0); they were fed for 05 weeks. Fries reared in T1, T2, T3 and T4 were fed with 75%, 50%, 25% and 0% dry feed respectively, while those in T0 were fed with 100% dry feed. All four treatments contain macroinvertebrates produced in mass. The physicochemical parameters of the water, the survival and growth parameters of the fry were assessed. The results showed that the physicochemical parameters of the water were within the recommended standards for the culture of the majority of aquatic species The average final weight and specific growth rate of the fries were highest in T0 (11.95 \pm 0.06 g and 8.96 \pm 0.014 %.d⁻¹), followed by T1 (10.50 \pm 0.8 g and 8.58 \pm 0.2 % d⁻¹) and T2 (10.16 \pm 0.50 g and 8.49 \pm 0.14 % d⁻¹). They are very low in T4 (01.35 \pm 0.11 g and 2.72 \pm 0.23 %.d⁻¹). The specific growth rate and survival rate of T1, T2 and T0 were not significantly different (p > 0.05). T1 and T2 will therefore reduce the amount of artificial feed to be distributed; however, the optimal rate was 50% (T2). It is therefore possible to reduce the cost of fish products in a semi-intensive system.

Keywords: Fish farming; artificial feed; macroinvertebrates; supplementation; production; pig dung.

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1. Introduction

Aquaculture production in the world plays an increasingly important role in the food and nutritional security of populations in developed and developing countries, as aquaculture has significant potential to feed a growing world population [1,2]. Indeed, over the last ten (10) years global aquaculture has grown from 77.9 million tonnes (in 2010) to 122.6 million tonnes (in 2020). Conversely, during the same period, catches of fishery products as a whole have remained stable at around 92 million tonnes [3]. Despite the continuous and spectacular growth of aquaculture in most regions of the world, it remains underdeveloped in Africa and then in Sub-Saharan African countries in general, with respectively 1.92% and 0.59% of total world production in 2020 [3]. Benin, in particular, with an annual production of 2,649 tonnes in 2021, is one of the Sub-Saharan African countries with low fish production [4].

Despite the many efforts of various actors (State, donors, NGOs, researchers, producers, etc.) to promote this type of farming, aquaculture, which is a reliable means to fill Benin's needs for fish products, does not yet allow for a reduction in the import of frozen products, even though the country has significant water resources, constituting an important potential for its development [1,5,6]. Beninese aquaculture still faces serious problems [7-9]. Apart from environmental factors, one of the major constraints to the development of fish farming in Benin is the high cost of production and therefore the cost of fish products, which depends mainly on feed [3,8,10, 11].

Indeed, in aquaculture, feed represents an important part (50 to 70%) of the fish production cost [12]. The economic interest of this type of farming is therefore highly dependent on the availability and cost of feed [13,14]. Thus, reducing feed costs, and consequently controlling the cost of production of farmed fish, is one of the priorities in aquaculture [15]. This reduction in feed costs involves not only the availability of good quality, low-cost feed but also the reduction of the artificial feed distributed amount. Studies have addressed the issue of availability of good quality, low cost feed from a socio-economic perspective [16-18]. Furthermore, other studies have only looked at the alternatives of using harvest by-products as a feed base for fish farming, replacing fishmeal with other animal protein sources, their distribution frequencies and co-cultivation of fish [19-23]. Thus, very few studies have focused on reducing the amount of artificial feed to complement natural feed in livestock structures. However, several living organisms, which constitute a natural food for fish, are used in fish farming. These include phytoplankton, zooplankton, insects and some plants [24-26]. Among insects, macroinvertebrates are the main food source for several fish species and their use is essential for successful post-larval and juvenile rearing [27, 28]. Indeed, benthic macroinvertebrates play an important role in the aquatic food chain, as they are part of the aquatic organisms serving as live food for other living beings (fish, insects, amphibians...) found in aquatic ecosystems and their larvae constitute one of the excellent basic food products in the ration of almost all carnivorous fish fry [29-31]. Thus, the use of macroinvertebrates will play a very important role in the development of aquaculture in Benin, not only in terms of economic optimization but also in achieving adequate growth and survival performance. In this respect, the present study focuses on the use of artificial feed as a feed supplement while valorizing the live feed present in aquaculture ecosystems, in particular macroinvertebrates, produced with pig dung, in the rearing of catfish fry. The aim is to compare the survival and growth performance of Clarias gariepinus fry fed with benthic macroinvertebrates produced from

pig dung and artificial feed in different proportions to those of fry of the same species fed only with artificial feed, in order to determine the optimal artificial feed ration to be distributed as a supplement to these fry reared in a semi-intensive system.

2. Materials and methods

2.1. Experimental design

The experimental set-up consisted of 15 plastic buckets of 80 liters capacity, placed in the open air on the research station on fish farming diversification of the Research Laboratory on Wetlands (LRZH) of the University of Abomey-Calavi (UAC). These buckets were grouped into four treatments (T1, T2, T3 and T4) and a control (T0) which were repeated three (03) times. The buckets of these 4 treatments contain benthic macroinvertebrates (molluscs and Chirominidae) which were mass produced in them; they were made up of 10 dm³ of substrate (mixture of dry pig dung and sand) then 20 L of water [32,33]. While the buckets in the control area (T0) contain only 20 L of borehole water. Five (05) week old fries of Clarias gariepinus with initial weight of 0.52 ± 0.15 g were acclimatized for 48 h and were distributed in the 15 plastic buckets with a density of 0.6 ind.L⁻¹ and then fed for 05 weeks. Fries reared in treatments T1, T2 and T3 were respectively fed 75%, 50% and 25% of their feed ration in dry feed (coppens: 49% protein). Those in treatment T4 received no dry feed (0%), while the controls (T0) were fed 100% of their feed ration in dry feed. The artificial feed was fed at a ration rate of 7% of the total biomass for the first three (03) weeks and then at a rate of 5% until the end of the experiment [34]. The ration was distributed three times a day (9 am, 1 pm and 5 pm). Fertilization was renewed with one third of the initial dose of pig dung [32,33] every seven (07) days in the treatments T1, T2, T3 and T4, whose area receive in addition, a contribution of macroinvertebrates harvested in other buckets of massive and continuous production of these macroinvertebrates. Similarly, half of the water in all the rearing environments is renewed with borehole water.

2.2. Measurement of the water physicochemical parameters

During the experiment, the physicochemical parameters (pH, temperature and dissolved oxygen) of the water in the fries rearing buckets were measured in situ once a week. The pH and temperature were measured with a W340i multiparameter conductivity meter. Dissolved oxygen was measured with a HANNA oximeter (HI 9143 Microprocossor Auto Cal Dissolved Oxygen Meter). Various chemical analyses of the water in each production environment were then carried out using 500 mL of water taken from plastic bottles (0.5 L capacity). Thus, ammonium, nitrate, and nitrite were respectively determined by the Nessler-380, Cadmium-335 reduction and Diazotation-371 methods with the HACH spectrophotometer).

2.3. Fries growth and survival rate

Growth monitoring of fries was carried out every 07 days; they were weighed, after a quick wipe on a towel to remove body water weight, with a Proscale - HC-600AX precision electronic balance, sensitive to 0.01g. Similarly, a systematic count of all individuals was carried out to assess fries survival. Dead fries were removed daily and counted.

The different survival and growth parameters calculated for each treatment are

- Survival rate TS in %.

$$TS = 100 \text{ x Nf/Ni}$$
(1)

With Ni = initial number of individuals and Nf = number of individuals at the end of the experiment

- Daily weight gain (DWG) in g/d

$$YDG = (Pf-Pi)/t$$
 (2)

With Pi = initial weight, Pf = final weight, and t is the duration in days.

- Specific Growth Rate TCS in %.d⁻¹

$$TCS = 100[Ln (Pf)-Ln(Pi)]/t$$
 (3)

<u>With</u> Ln = natural logarithm and t = duration in days

2.4. Statistical analysis

The statistical analysis of the results obtained was carried out using SAS statistical software version 9.4 by the method of analysis of variance with one classification criterion (ANOVA I) at a threshold of 5%. Fisher's LSD (Least Significant Difference) was used to compare the different means.

3. Results

3.1. Physicochemical parameters

The average values of the physicochemical parameters of the rearing area of the *Clarias gariepinus* fries of the different treatments are summarized in Table 1. According to Table 1, the temperature and pH mean values evolution during the experiment did not fluctuate much; the mean temperature of all rearing area was 29.86 \pm 0.90 °C and the mean pH was 6.94 \pm 0.75; there was no significant difference (p > 0.05) between the different rearing areas for these two parameters. The average concentrations of dissolved oxygen were significantly higher in the unfertilized/control environments (6.17 \pm 0.69 mg.L⁻¹) than in the fertilized environments (T1, T2, T3 and T4). In fact, the average dissolved oxygen concentrations of the latter are higher in treatments T2 (4.67 \pm 0.68 mg.L⁻¹) and T4 (4.61 \pm 0.73 mg.L⁻¹) with a significant difference (p < 0.05) compared to the area in treatments T1 (4.59 \pm 0.76 mg.L⁻¹) and T3 (4.48 \pm 0.87 mg.L⁻¹). The mean values of dissolved salts (NH₄⁺, NO₃⁻ and NO₂⁻) are significantly higher (p < 0.05) in the fertilized area compared to the control area (T0), which were not fertilized and where they are very low. In fact, the average values of NH₄⁺ and NO₃⁻ are higher in the area of treatments T2 and T4 with a significant difference (p < 0.05) compared to those of treatments T1 and T3. The average value of NO₂⁻ for the four treatments is 0.0613 \pm 0.022 mg.L⁻¹; there is no significant difference (p > 0.05) between these treatments.

Parameters	Т0	T1	T2	Т3	T4
Temp (°C)	$29,87 \pm 0,83^{a}$	29,85 ± 0,91 ^a	$29,88 \pm 0,89^{a}$	$29,89 \pm 0,93^{a}$	$29,83 \pm 0,95^{a}$
рН	$6,91 \pm 0,21^{a}$	$6,96 \pm 0,25^{a}$	$6,93 \pm 0,20^{a}$	$6,95 \pm 0,18^{a}$	$6,94 \pm 0,24^{a}$
DO (mg.L ⁻¹)	$6, 17 \pm 0,69^{a}$	$4{,}59\pm0{,}76^{b}$	$4,67 \pm 0,68^{\circ}$	$4,\!48\pm0,\!87^{\mathrm{b}}$	$4,61 \pm 0,73^{\circ}$
NH_4^+ (mg.L ⁻¹)	$1,47 \pm 0,53^{a}$	$13,95 \pm 7,63^{b}$	$14,13 \pm 6,95^{\circ}$	13,97 ± 8,93 ^b	$14,31 \pm 7,86^{c}$
$N0_{3}^{-}$ (mg.L ⁻¹)	$5,36 \pm 1,29^{a}$	10,63 ± 5,81 ^b	$11,09 \pm 6,93^{\circ}$	$10,86 \pm 8,53^{\rm b}$	11,13 ± 7,23 ^c
$N0_2^{-1}$ (mg.L ⁻¹)	0,0473 ± 0,011 ^a	0,0609 ± 0,019 ^b	0,0619 ± 0,019 ^b	$0,0621 \pm 0,034^{b}$	$0,0602 \pm 0,017^{b}$

Table 1: Mean \pm Standard deviation \pm 95% IC of physicochemical characteristics of the water in the different
treatments.

Legend: Values in the same line marked with the same letter do not differ significantly (P > 0.05).

Temp = Temperature; DO = Dissolved Oxygen; NH_4^+ = ammonium; NO_3^- = nitrate; NO_2^- = nitrite.

3.2. Fries survival and growth parameters

Figure 1 shows the survival rate, mean final weight and specific growth rate of *Clarias gariepinus* fries by treatment. According to figure 1a, the survival rate of *Clarias gariepinus* fingerlings is higher in the control (T0) and T3 treatment ($88.88 \pm 4.81\%$) which are fed 100% and 25% of their ration in dry feed respectively; followed by fries from treatments T1 and T4 (86.11 \pm 4.81%) which are fed at 75% and 0% of their ration in dry feed respectively and finally those from treatment T2 ($83.33 \pm 8.33\%$) which are fed at 50% of their ration in dry feed. However, there was no significant difference between these different survival rates (P > 0.05). The average final weight of the fries was significantly higher (p < 0.05) in the control area (11.95 \pm 0.06 g) compared to the other (fertilized) area (figure 1b). This is followed by the fries in treatments T1 and T2 (10.50 \pm 0.8 g and 10.16 \pm 0.50 g respectively) which were significantly different (p < 0.05) from the other fertilized environments (T3 and T4). The average final weight of the fries was low in treatment T3 (04.57 \pm 0.33 g) and very low in treatment T4 (01.35 \pm 0.11 g); there was a significant difference (p < 0.05) between treatments T3 and T4. According to figure 1c, the specific growth rate (SGR) of the fries is higher in the control area (8.96 \pm 0.014 %.d⁻¹), followed by those of treatments T1 and T2 (respectively 8.58 ± 0.2 %.d⁻¹ and 8.49 ± 0.14 %.d⁻¹) which are not significantly different (p > 0.05) between them and the controls (T0). The lowest fries specific growth rate (SGR) was found in treatment T4 $(2.72 \pm 0.23 \text{ \%.d}^{-1})$ which is significantly different from the other fertilized area. The different survival and growth performances of the fries fed with 50% of their ration in dry feed (T2) were thus close to those of the fries fed with 75% of their ration in dry feed (T1) and were not significantly different (p > 0.05) between them. The survival rate and specific growth rate of these two treatments (T1 and T2) were not significantly different (p > 0.05) between them and between those of the control fries, fed with 100% of their dry feed ration (T0).





Figure 1: Mean ± Standard deviation ± 95%IC of Survival rate (a), average final weight (b) and specific growth rate (c) of *Clarias gariepinus* fries per treatment.

Legend: Bars with different letters are significantly different at the 5% level.

According to figure 2 which shows the evolution of the mean weights of *Clarias gariepinus* fries by treatment as a function of time, the mean weights of *Clarias gariepinus* fries fed with dry feed as a supplement (T1, T2 and T3) or not (T0) increased linearly throughout the experimental period, in contrast to those of the fries that did not receive dry feed (T4). Indeed, during the first two weeks of rearing, the fries from T0, T1, T2 and T3 have almost the same body weight, which was higher than that of the T4 treatment (25% of dry feed). But after the two weeks, the average weight of the fries fed only (100%) with dry feed (T0) was higher than that of the fries in treatments T1 and T2 until the end of the experiment. After the third week of rearing, the weight of the fries fed with 75% dry feed (T1) became slightly higher than that of the fries fed with 50% dry feed (T2) until the end of the experiment.





4. Discussion

4.1. Physicochemical parameters

The physicochemical parameters of the water in the fries rearing environments were in conformity with the values allowing the survival and growth of the catfish *Clarias gariepinus* fries. Indeed, *C. gariepinus* survives and grows best in waters with a pH between 6 and 9; because if the pH is outside this range, the growth of the fish is reduced [35,36]. References [37,38] report that the optimal temperature for growth of *C. gariepinus* is between 28 and 30°C. Similarly, this species adapts well to extreme environmental conditions and can live when dissolved oxygen levels are 3.5 mg.L⁻¹ or higher [39,40]. Oxygen levels in our fertilized environments were similar to those obtained in ponds fertilized with pig dung by [41].

4.2. Fries survival and growth parameters

The very low specific growth rate and final average weight observed in *Clarias gariepinus* fries fed only with macroinvertebrates (T4 / 0% dry feed) shows that dry feed, distributed as a supplement, is essential for a good growth performance of these fries in semi-intensive rearing. The survival rates of the fries in the different rearing area, which are plastic buckets during our experiment, are lower than those obtained by [41] which were $92.3\pm5.0\%$ after fertilizing the ponds with pig manure. This difference can be explained by the rearing environments nature and surface area. Indeed, the buckets surface area that was small may favour cannibalism. However, the specific growth rate recorded in the different treatments (T1, T2, T3 and T0,) of our experiment is higher than the one obtained by [41] which was $0.061\pm00\%$.d⁻¹ after fertilizing the ponds with pig manure. This difference is due to the additional feed provided to the fries in our rearing environments. The final average weight of *Clarias gariepinus* fingerlings fed only artificial feed (T0) in our work is slightly lower than that of fingerlings of this species (12.90g) fed dry feed (imported + local) in semi-intensive pond rearing [33] with an initial average weight of 4.1g per individual. This difference would be due to the low initial weight of the fries used in our study.

The growth performance of fries fed with 25% dry feed (T3) was poor compared to T1 and T2 fries fed with 50% and 75% dry feed respectively, which all showed good growth performance. This difference might be explained by the supplementary feed ration inadequacy.

The specific growth rates recorded in treatments T1 and T2 and in the control (T0) during our experiment were not significantly different (p > 0.05) and were in accordance with the norms, as *Clarias gariepinus* fries weighing between 0.5 and 10 g had specific growth rates between 8 and 12 %.d⁻¹ when the temperature was around 30°C [42,43]. Furthermore, there was no significant difference (p > 0.05) between most survival and growth parameters of the fries in the control (T0) and in these two treatments (T1 and T2). These latter will therefore reduce the dry feed amount to be fed to the *C. gariepinus* fries reared in the continuous macroinvertebrate production area and will result in good growth and survival of the catfish. However, it is the T2 treatment (50% dry feed) that is the optimal treatment because it allows to reduce considerably the feed used amount and to have an appreciable result; moreover, the dissolved oxygen level of the water of this treatment is significantly higher than that of the T1 treatment.

5. Conclusion

The parameters of the fry rearing waters have been included in the range of standards recommended for the culture of the majority of aquatic species. The growth parameter values of the fries in treatments T1 (75%) and T2 (50%) were significantly better than those in treatments T3 (25%) and T4 (0%). These first two treatments will therefore reduce the amount of feed to be distributed and provide good growth and survival performance of *Clarias gariepinus* catfish reared in a semi-intensive system. The optimal treatment is therefore T2; thus, the optimal rate of dry feed ration distributed as a supplement to these *Clarias gariepinus* fries reared in continuous macroinvertebrate production environments is 50%. It is then possible to rear *Clarias gariepinus* with simple techniques adapted to rural conditions in order to allow fish farmers in developing countries to reduce the cost of production and consequently the cost of fish products.

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