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OPTIMIZATION OF ZOOPLANKTON PRODUCTION FROM PIG DUNG OPTIMAL DOSE: NON-RENEWED MEDIUM

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ABSTRACT

Present study was carried out for the optimization of a non renewed medium for the production of zooplankton from pig dung. Study was carried out in plastic buckets with two treatments (T<sub>1</sub> and T<sub>2</sub>) and one control for 27 days. Each treatment was replicated thrice. The buckets were seeded with zooplankton with an initial density of 37 individual/l (Day<sub>0</sub>). Evaluation of zooplankton density in the experimental bucket was carried out on the interval of each three days up to the completion of the study. The results of the study revealed that the fishing followed with a periodical fertilization improved the zooplankton production ( $p < 0.05$ ) with rotifers predominance. Thus the treatment T<sub>2</sub> media offered the best zooplanktonic average density ( $896 \pm 977 \text{ ind.l}^{-1}$ ) as compared to the treatment T<sub>1</sub> ( $248 \pm 129 \text{ ind.l}^{-1}$ ). The adoption of this production technique of zooplankton permitted to reduced production time of this small sized live prey which could be maintained during several weeks for the aquaculture hatcheries.

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## 1 Introduction

The main constraint that hinders the aquaculture development in developing countries is the rearing of domesticated fish species larvae. Success of the rearing is particularly depends on the feeds of larvae so as to make fingerling available to the pisciculturist (Brunson et al., 1999). Most effective aliment for the fish larvae is the zooplankton (live food), as dry food (inert) have shown their limit (Curnow et al., 2006; Hamza et al., 2007). Occurrence of catfish larvae having a small vitellus that is rapidly resorbed (Legendre et al., 1992) and its survival is limited in absence of the appropriate live food (Otémé & Gilles, 1995).

*Artemia* sps are the organisms which mostly used as a live prey feed (Awaiss, 1992). But utilization of these cysts, mostly in rural tropical area is difficult because of constraining hatching conditions, its high cost and unavailability on the local market (Agadjihouèdé et al., 2012). So, the utilization of other local live prey with a high potential of production is an alternative.

As an alternative, periodical harvesting of the zooplankton population and the use of specific seaweed culture allows the continuous mass production of these local zooplanktons in the hatcheries (MacIsaac & Gilbert, 1989). These methods are not only required a wide space and expertise for the large scale production of zooplankton but also very costly (Park et al., 2001).

Furthermore, the monospecific culture has shown their limitation by the presence of unwanted organisms (Saint-jean et al., 1994; Awaiss & Kestemont, 1997). It is therefore essential to develop a plurispecific production media for the mass of the zooplankton. In this context, media contains one of the phytoplankton along with animal dejections which are easily available and accessible to the fish farmer are reported beneficial for the zooplankton culturing.

In contrast to the monospecific culture media with animal dejection, plurispecific media with animal dejection including pig dung not found significantly superior for the mass production of fresh water zooplankton but the use of optimal dose pig dung in an plurispecific media for the production of zooplankton is well reported, in fact, such a rearing media is well known for increasing the rate of zooplankton population dynamics for a short period which was followed by the fall in production with the optimal dose of 600 g.m<sup>-3</sup> of dry pig dung (Akodogbo et al., 2014a, Akodogbo et al., 2014b). This study was planned for the optimization of continuous and mass plurispecific production of local zooplankton with pig dung, through the dynamic of production with the fishing technique followed by the periodical fertilization. It will favor the decrease of fish larvae production costs and allow the rural pisciculturists to ensure a continuous production of the local

live food (zooplankton) and a good fish larvae development without rupture food.

## 2 Materials and Methods

### 2.1 Experimental design

The experiment was conducted in the nine plastic buckets with 80 liters capacity at wetlands research station, University of Abomey-Calavi, Benin. Two treatments (T<sub>1</sub> and T<sub>2</sub>) and a control (T<sub>0</sub>) were established in triplicates. The buckets of treatment T<sub>1</sub> were fertilized once only whereas the buckets of the treatment T<sub>2</sub> were periodically fertilized in addition to the initial dose during the complete study periods. Moreover, the zooplankton population of these medium was partially fished periodically.

While the treatment T<sub>0</sub> has not received any type of fertilizers. Before starting the study all the buckets were washed with bleaching water and dried for 24 hrs. This was followed the addition of 40 liters of drilling water. Immediately after this, buckets of the treatments T<sub>1</sub> and T<sub>2</sub> were fertilized by dry pig dung of 600 g.m<sup>-3</sup> while the T<sub>0</sub> remained unfertilized (Akodogbo et al., 2014b).

Three days later, all the buckets were seeded by phytoplankton filtered through 50 µm silk cloth along with 10 liters green water of pond. According to Guiral et al. (1994) three days period is enough for the establishment of phytoplankton. So, after three days of phytoplankton inoculation all the buckets were seeded by 37 ind.l<sup>-1</sup> (7 ind.l<sup>-1</sup> of rotifers; 28 ind.l<sup>-1</sup> of copepods and 2 ind.l<sup>-1</sup> of cladocerans) zooplankton population (D<sub>0</sub>) which was obtained with the help of 50 µm plankton net. From D<sub>12</sub>, the fertilization has been renewed with the one third (1/3) of the initial dose at each three days interval in the treatment T<sub>2</sub> buckets that have been partially fished (50%) of its population before the other fertilization (Kabir et al., 2010).

### 2.2 Zooplankton Production Follow-up

Observation of Zooplankton multiplication was started from the D<sub>7</sub> and continued up to the 27<sup>th</sup> days each after three days intervals (Kabir et al., 2010). For this, 5 liter water sample were harvested from each bucket and passed from the 50 µm silk cloth for the calculation the harvested population of the zooplankton population.

Isolated filtrate was fixed with 5% formaldehyde. From this fixed sample, a adequate samples were pipette out in 1000 µl Eppendorf bottle and observed under a light microscope (PIERON, S/N S294452/ X 4). The present zooplankton populations were enumerated to evaluate the densities (D) of different type of zooplankton groups.

The daily production (P), the intrinsic increase rate (Kr) and the doubling time (Td) of the zooplankton population were calculated from the following formula:

$$-D = (N/V_1) \times (V_2/V_3);$$

$$-P = (N_t - N_0)/t;$$

$$-Kr = (\ln N_t - \ln N_0)/t;$$

$$-Td = \ln 2/Kr; \text{ (James et al., 1986).}$$

Whereas, N = number of individuals counted in an under-sample; V<sub>1</sub> = observed volume (under-sample); V<sub>2</sub> = concentration volume; V<sub>3</sub> = filtered water volume; N<sub>t</sub> = final number per liter; N<sub>0</sub> = initial number per liter and t = production time.

### 2.3 Measurement of physico-chemical and trophic parameters

The physical and chemical parameters of the cultural medium (temperature, pH, conductivity and dissolved oxygen) were *in situ* measured. Diverse chemical analyses were carried out for each bucket, for this 500 ml of water sample were collected in plastic bottles. From the collected samples concentration of ammonium, nitrates, nitrites and phosphates were measured by the Nessler-380 methods. Measurement of Cadmium-335 reduction to Diazotation-371 and to Phosver 3-490 with the spectrophotometer HACH.

Similarly, 500 ml water sample has been drawn into other plastic bottles (0.5 l of capacity) from each bucket and measure the content of chlorophyll *a* (trophic parameter) which allowed to appreciate the phytoplankton quantity. Each water sample bottle was packed inside aluminum paper to prevent sample photosensitivity. The chlorophyll *a* measurement has been achieved by spectrophotometer according to Pechar (1987) method.

### 2.4 Statistical analyzes

The statistical analysis of obtained results was performed with statistic logical SAS version 9.2 by analysis of variance method with one classification criteria (ANOVA I) (Scherrer, 1984). The LSD (Least Significant Difference) of Fisher (Saville, 1990) was used to compare the different average.

## 3 Results

### 3.1 Physico-chemical and trophic parameters

Table 1 summarizes the physico-chemical and trophic mean values of different treatments. According to the Table 1, the conductivity and average concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> were higher in the treatment T<sub>2</sub> buckets (periodical fishing and fertilization). The variance of analysis with one classification criteria (ANOVA I) were applied to the different parameters and result revealed a significant differences of conductivity, ammonium, nitrates, phosphate and dissolved oxygen rates between the different treatments (p < 0.05). But the difference was not significant for the temperature, the pH and the nitrites between these treatments (p > 0.05).

Like other parameters, average chlorophyll *a* concentration (Table 1) was higher for the treatment T<sub>2</sub> buckets. The variance of analysis with one classification criteria (ANOVA I) applied to the different values of chlorophyll *a* concentration revealed significant difference between these treatments (p < 0.05). Amount of chlorophyll *a* increased with the increasing time duration in treatment of T<sub>2</sub>. On the other hand, this chlorophyll concentration has reached to its peak on D<sub>15</sub> in the treatment T<sub>1</sub> medium (Figure 1).

Table1 Physico-chemical characteristics and chlorophyll *a* concentration of different treatments.

	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>
Temperature (°C)	31.10 ± 0.66 <sup>a</sup>	31.21 ± 0.59 <sup>a</sup>	31.28 ± 0.70 <sup>a</sup>
pH	6.18 ± 0.41 <sup>a</sup>	6.16 ± 0.33 <sup>a</sup>	6.16 ± 0.43 <sup>a</sup>
Dissolved Oxygen (mg.l <sup>-1</sup> )	5.36 ± 0.20 <sup>a</sup>	5.57 ± 0.31 <sup>b</sup>	5.72 ± 0.41 <sup>c</sup>
Conductivity (µS.cm <sup>-1</sup> )	71.90 ± 4.01 <sup>a</sup>	127 ± 7.03 <sup>b</sup>	151.99 ± 35.92 <sup>c</sup>
NH <sub>4</sub> <sup>+</sup> (mg.l <sup>-1</sup> )	0.13 ± 0.05 <sup>a</sup>	0.44 ± 0.27 <sup>b</sup>	1.03 ± 0.50 <sup>c</sup>
NO <sub>2</sub> <sup>-</sup> (mg.l <sup>-1</sup> )	0.007 ± 0.003 <sup>a</sup>	0.010 ± 0.005 <sup>a</sup>	0.011 ± 0.003 <sup>a</sup>
NO <sub>3</sub> <sup>-</sup> (mg.l <sup>-1</sup> )	4.99 ± 1.38 <sup>a</sup>	6.46 ± 4.19 <sup>b</sup>	11.06 ± 4.90 <sup>c</sup>
PO <sub>4</sub> <sup>3-</sup> (mg.l <sup>-1</sup> )	1.23 ± 0.6 <sup>a</sup>	7.94 ± 1.67 <sup>b</sup>	12.56 ± 7.90 <sup>c</sup>
Chlorophyll <i>a</i> (µg.l <sup>-1</sup> )	95.02 ± 57.83 <sup>a</sup>	272.95 ± 157.73 <sup>b</sup>	456.78 ± 178.07 <sup>c</sup>

The values affected with the same letter in exponent on the same line were not significant different (p > 0.05).

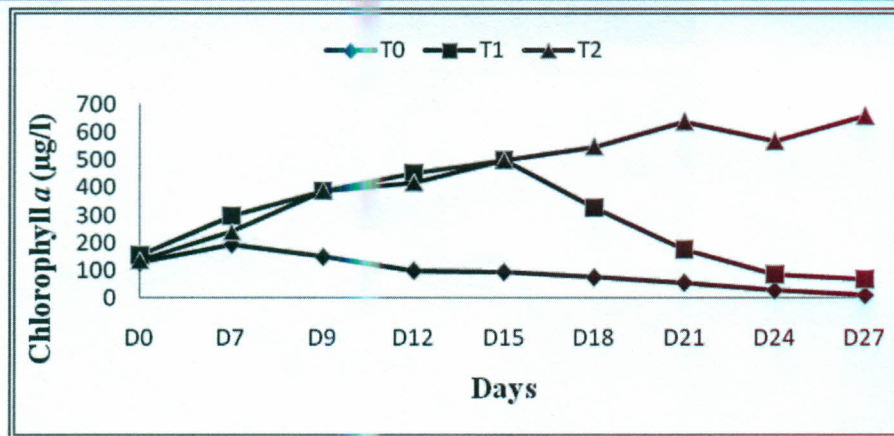


Figure 1 Evolution of chlorophyll *a* concentration of different treatments in function of time.

### 3.2 Variation of zooplankton densities

The analysis of Figure 2 showed that average zooplankton densities during the experimentation are higher for the treatment T<sub>2</sub> bucket ( $896 \pm 977$  ind.l<sup>-1</sup>) it was followed by those of T<sub>1</sub> ( $248 \pm 129$  ind.l<sup>-1</sup>).

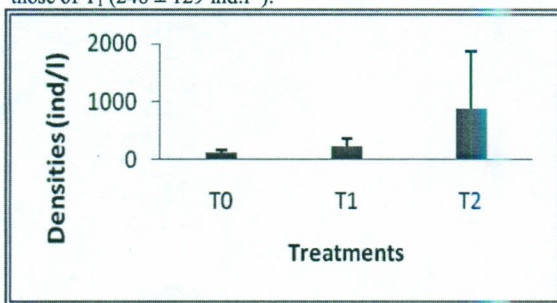


Figure 2 Zooplankton total average densities by treatments.

The variance of analysis with one classification criteria (ANOVA I) revealed significant differences between total average zooplankton densities in the difference treatments ( $p < 0.05$ ).

Results presented in figure 3 revealed the total zooplankton densities with the time. Furthermore, the average zooplankton density in treatment T<sub>1</sub> increased progressively from D<sub>0</sub> to D<sub>15</sub> where it has reached to its peak (413 ind.l<sup>-1</sup>). Similarly, for the bucket of the treatment T<sub>2</sub> has also shown progressive increases from D<sub>0</sub> to D<sub>15</sub> (444 ind.l<sup>-1</sup>).

But from D<sub>15</sub> to D<sub>27</sub> an exponential improvement was reported in the zooplankton density and it increased from 444 ind.l<sup>-1</sup> to 2929 ind.l<sup>-1</sup>. From D<sub>15</sub> to D<sub>27</sub> the daily production, intrinsic increase rate and doubling time of zooplankton population for treatment T<sub>2</sub> were 207 ind.l<sup>-1</sup>.d<sup>-1</sup>; 0.16 in 24 h and 4.41 days respectively while a reduction was reported in the bucket containing treatment T<sub>1</sub> during this period. Furthermore, the daily production of zooplankton for this period was 7.6 times higher than the D<sub>0</sub> to D<sub>15</sub> (27 ind.l<sup>-1</sup>.d<sup>-1</sup>).

The analysis of Figure 4 showed that the average densities of different zooplankton groups (rotifers, copepods and cladocerans) are higher in T<sub>2</sub> medium.

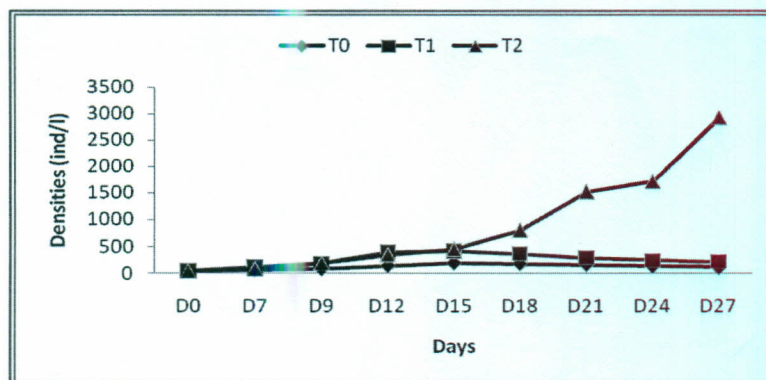


Figure 3 Evolution of total zooplankton average densities of different treatments in function of time.

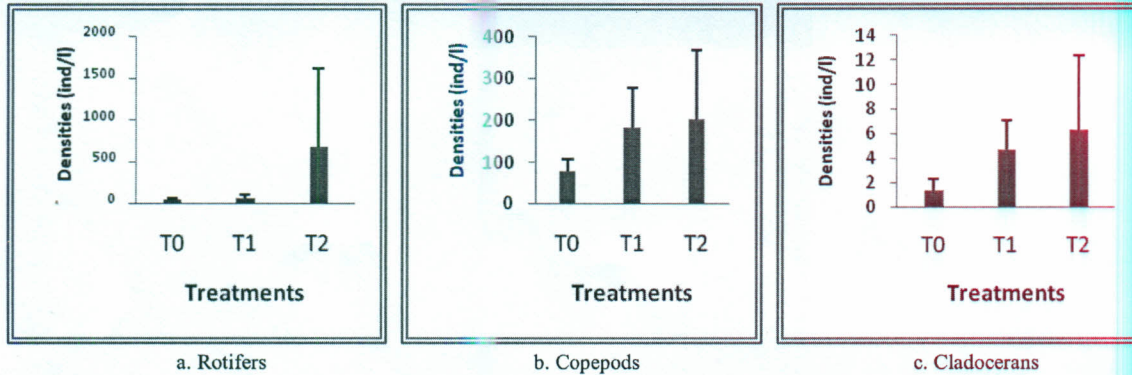


Figure 4 Different zooplankton groups' density by treatments.

Furthermore, the culturing medium of T<sub>2</sub> were favored the growth of rotifers ( $687 \pm 941 \text{ ind.l}^{-1}$ ) which was followed by the copepods ( $202 \pm 161 \text{ ind.l}^{-1}$ ) whereas the medium of the treatment T<sub>1</sub> was favorable for the copepods ( $182 \pm 91 \text{ ind.l}^{-1}$ ) and it was followed by the rotifers ( $61 \pm 39 \text{ ind.l}^{-1}$ ).

#### Discussion

The results of the study revealed that the average temperature of buckets water was around  $31.20 \pm 0.09^\circ\text{C}$  during the study time, this temperature favored the production of high density of rotifers i.e. *Brachionus calyciflorus* (28 and  $32^\circ\text{C}$ ) in the fresh water culture, (Kabir et al., 2010). The conductivity and the average concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{PO}_4^{3-}$  were the highest in bucket containing T<sub>2</sub> medium it may be because of the continuous periodical addition of the fertilizer during the experimentation. The periodical application of pig dung has significantly improved the dissolved salts rates in the water and it change the physico-chemical quality of the water (Adedeji et al., 2011). Results of the study confirmed the positive effect of pig dung on the nutritious quality of water (Akodogbo et al., 2014a). Progressive decrease in the concentration of chlorophyll a was noticed, in the treatment containing medium T<sub>1</sub> from D<sub>15</sub> onward, it may be due to the exhaustion of the culture medium, during the time, in nutritious salts (Agadjihouédé et al., 2010a; Agadjihouédé et al., 2010b; Akodogbo et al., 2014a).

On the other hand, in the treatment T<sub>2</sub>, continuous amelioration in the rate of chlorophyll a and phytoplankton population from D<sub>15</sub> to the completion of the study, it may be due to the periodical supply of fertilizers which liberated nutritious salts required for the growth and development of phytoplankton. This confirmed that the development of phytoplankton depends on nutritious salts (Schlumberger & Bouretz, 2002; Akodogbo et al., 2014a). The fall in the zooplankton density in treatment medium T<sub>1</sub> after D<sub>15</sub> can be explained by the reduction in phytoplankton density because of the zooplankton peak coincided with the phytoplankton one. This proved zooplankton dependence towards phytoplankton which constitutes their food (Seyer, 2002). This reduction of zooplankton density was also due to the exhaustion of the fertilizers in nutritious substances required for the

phytoplankton development. As in 20 days, the organic material was completely mineralized in the water (Berard 1993). These results justified a positive correlation between the nutritious salts, phytoplankton population and the zooplankton (Lazzaro & Lacroix, 1995; Akodogbo et al., 2014a). Likewise, the fall of this density on D<sub>15</sub> confirmed by the works of Akodogbo et al. (2014b) which showed that the maintain time of the pig dung optimal dose is 14 days.

The increasing of total zooplankton density in the treatment T<sub>2</sub> medium after D<sub>15</sub> was due to the improvement in phytoplankton density inside this medium. The food (phytoplankton) was therefore permanently available for the zooplankton, hence their good growth. Improvement in the phytoplankton density was due to the periodic fertilization in this culture medium which brought continuity in the availability of nutritious salts. Periodic fertilization from D<sub>12</sub> has then permitted to maintain optimal the zooplankton density so as to avoid the sudden death of the population comparatively to the treatment T<sub>1</sub> where in absence of fertilization, the zooplankton population density fell quickly for the lack of food. The latter was dominated by the copepods (Arimoro, 2006).

The partial and periodic harvest of the population has improved the total zooplanktonic density in treatments T<sub>2</sub> medium, which was due the rotifers preponderance. That eliminated of zooplankton population by the process of harvesting reduced the competition between the population which help in the raising of the population. This has permitted rotifers development which were small size species (Gilbert, 1988; Maclsaac & Gilbert, 1989). So, it's elimination from the production medium favor the older individuals who have a low fecundity rate and the development of amictic females. In fact, the age of the rotifers parental females influenced significantly the fecundity rate which linearly decreased according to the age. The amictic females have a very high reproductive rate as they are parthenogenetic individuals (Pourriot, 1983). This confirmed the high production of rotifers population in the treatment T<sub>2</sub> during the harvest. With respect to zooplankton production dynamic, the intrinsic increasing rate of zooplankton population in treatment T<sub>2</sub> (0.16 in 24h) was inferior to the one obtained (0.53 in 24h) by Awaïf &

Kestemont (1997). Similarly, the doubling time (4.41 days) was longer than the one obtained these latter (1.29 days). That difference was due to the fact that these authors have done monospecific culture of amictic young females of rotifers (*Brachionus calyciflorus*) fed with specific seaweed (*Dictyosphaerium chlorelloides*), whereas in present study the culture medium T<sub>2</sub> were plurispecific in phytoplankton and in zooplankton. This confirmed the studies of Agadjihouédé et al. (2011) who showed that the zooplankton increase rate is higher during the monospecific production than multispecific ones.

### Conclusion

Plurispecific production of zooplankton based on pig dung could be optimized as non-renewed medium through the continuous fertilization and the partial fishing of zooplankton which avoided the reduction in the zooplankton population and improved rotifers production. The periodical fertilization helps in continuous food supply (phytoplankton) to the zooplankton whose partial harvest eliminated from culture medium a part of the big size organisms of which some are in food competition (cladocerans) with the rotifers and others were their predators (copepods). The adoption of this production technique in continuous and mass production of the zooplankton permitted to get in a short time satisfactory population densities which could be maintained during several weeks. It allowed then the small size live prey production for the for the aquaculture hatcheries.

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