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Optimization of zooplankton production from pig dung optimal dose: renewed medium

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ABSTRACT

Present study was realized in the aim to optimize of a renewed medium for the production of zooplankton with pig dung. In fact, the experiment was carried out in triplicate in plastic buckets, grouped together by three treatments (T_1, T_2 and T_3) which were fertilized and a control (T_0) during 27 days. The buckets were seeded with zooplankton with an initial density of 37 individual/l (Day_0). From D_{12}, those of T_2 and T_3 have sustained a partial and periodical renewal (50%) in the production medium. The treatment T_3 was, in addition, fertilized periodically. The zooplankton density evolution is followed up through a sampling, every three days. The results have shown that the renewal followed with a periodical fertilization improved the zooplankton production ($p < 0.05$) with rotifers predominance. Thus the treatment T_3 media offered the best zooplanktonic average density (631 ± 440 ind/l) as compared to the treatment T_2 (392 ± 253 ind/l). That renewal prevents the pollution of and the congestion of production medium. The adoption of this production technique of zooplankton permitted to get small sized live prey in mass which could be maintained during the period of larval rearing for the aquaculture hatcheries.

1. Introduction

The bottleneck of most of the fresh water pisculturists, in developing countries, is the obtaining of sufficient fingerling, key for the aquaculture, by reason of their elevated death rate at the early step of life. Their production efficiency is hindered by the larvae feeding (Arimoro, 2006). Yet, the most effective aliment for the fish larvae, occurrence of catfish larvae, is the zooplankton (Legendre et al., 1992). Artemia are the organisms which mostly used as a live prey feed (Awaïss, 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, this increase the cost of fish production (Kestemont and Awaiss, 1989; Agadjihouèdé et al., 2012). So, the utilization of other live prey with a high potential of production, is an alternative. Then the intensive production of local planktonic organisms for the development of aquaculture is primordial.

Otherwise, techniques including renewal and periodical harvesting of the zooplankton population allow the mass production of the local zooplankton for the hatcheries (Arimoro, 2005). These productions are constraining, as for the choice of planktonic species cultivated as these culture are often monospecific. Likewise, the monospecific culture has shown their limit with the presence of unwanted organisms (Saint-Jean et al., 1994; Awaïss and Kestemont, 1997). It is therefore essential to develop a plurispecific production and mass of zooplankton which is less constraining with animal dejections which are accessible and available to pisculturists. In contrast to the monospecific culture with animal dejections, plurispecific culture of the fresh water zooplankton with animal dejections including the pig dung, has not reached a significant development. However, the dynamic of the zooplankton population produced with pig dung and the optimal dose of these dejections for a plurispecific production of the zooplankton are known. In fact, such a rearing media is well know an increase rate of short period followed by the fall in production with the optimal dose of 600 g/m^3 of dry pig dung (Akodogbo et al., 2014a, 2014b). Some problems subsist as for the obtainment of mass and durable plurispecific production.

This study was aimed at the optimization of mass plurispecific production of local zooplankton with pig dung, through the dynamic of production centered on the renewal of medium. It will favor the decrease of fish larvae production costs and allow the rural pisciculturists to ensure a mass production of the local live food (zooplankton) and a good fish larvae development without rupture food during the period of larval rearing.

2. Materials and methods

2.1. Experimental design

The experimental device was constituted of twelve (12) plastic buckets with 80 liters capacity, disposed in free air, at wetlands research station, University of Abomey-Calavi, Benin. These buckets were grouped into three triplicates of three treatments (T_1, T_2 and T_3) and a control (T_0). The buckets of treatment T_1 and T_2 were fertilized once whereas those of T_3 were periodically fertilized in addition to the initial dose during the complete study periods. Moreover, the production medium of treatment T_2 and T_3 were partially renewed periodically. Before putting the water in the buckets, these latter have been washed with bleaching water and dried for 24 hours. The following day, they have received 40 liters of drilling water. Immediately after this, buckets of the treatments T_1, T_2 and T_3 were fertilized by dry pig dung with a dose of 600 g/m^3 (Akodogbo et al., 2014b). Three (03) days after the fertilization, all the buckets were seeded with phytoplankton with 10 liters of pond water green enough filtered on a silk of 50 µm. Three days later (D_0), sufficient period to allow the growing of phytoplankton (Guiral et al., 1994), some zooplankton harvested in a pond, with a plankton net of 50 µm has been seeded in each bucket with an initial density of 37 ind/l (7 ind/l of rotifers; 28 ind/l of copepods and 2 ind/l of cladocerans). From D_{12}, the treatment T_2 and T_3 buckets were renewed at 50% with pond water filtered through a silk of 50 µm every three days (Saint-Jean et al., 1994). The fertilization has been renewed with the one third (1/3) of the initial dose at each three days interval in the treatments T_3 buckets (Saint-Jean et al., 1994; Kabir et al., 2010).

2.2. Zooplankton production follow-up

Zooplankton was sampled from the D_7, every three days (Kabir et al., 2010), until the 27th production days. In each bucket, 5 liters water sample were taken and then filtered through a silk of 50 µm for the zooplankton harvest; this filtrate was fixed with 5% formaldehyde. Some under-samples of this harvest were taken with an Eppendorf pipette (capacity: 1000 ml) and observed under a light microscope (PIERRON S/N S 294452 X 4). The present zooplankton organisms were enumerated to evaluate the densities (D) of the different 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:

- $D = (N/V_1) \times (V_2/V_3)$;
- $P = (N_t – N_0)/t$;
- $Kr = (\ln N_t - \ln N_0)/t$;
- $Td = \ln_2/Kr$; (James et al., 1986).

Whereas, N = number of individuals counted in an under-sample; V_1 = observed volume (under-sample); V_2 = concentration volume; V_3 = filtered water volume; N_t = final number per liter; N_0 = initial number per liter and t = production time.

2.3. Measurement of physico-chemical and trophic parameters

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

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

2.4. Statistical analyses

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) (Dagnelie, 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 Table 1, the mean temperature of water in buckets was around 31.07 ± 0.8°C. The pH mean values were around 6.05 ± 0.51 and slightly fluctuated. The conductivity and average concentrations of NH_4^+, NO_3^- and PO_4^{3-} were higher in the treatment T_3 buckets (periodical renewal and fertilization). The variance of analysis with one classification criteria (ANOVA I) applied to the different parameters and result revealed significant differences of conductivity, ammonium, nitrates and phosphates 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_3 buckets. The variance of analysis with one classification criteria (ANOVA I) applied to the different value of chlorophyll *a* concentration revealed significant differences between these treatments ($p < 0.05$). The evolution of the chlorophyll *a* concentration during the experimentation has shown that the culture medium of treatment T_2, T_1 and T_3 have reached their peak on 12th, 15th and 21st production days (Figure 1).

3.2. Variation of zooplankton densities

The analysis of Figure 2 showed that average zooplanktonic densities during the experimentation, were higher for the treatment T_3 medium (631 ± 440 ind/l), it was followed by the ones of T_2 (392 ± 253 ind/l). The variance of analysis with one classification criteria (ANOVA I) revealed a significant difference between total average zooplankton densities in the different treatments ($p < 0.05$).

Table 1
The Physico-chemical characteristics and chlorophyll *a* concentration of different treatments

	T0	T1	T2	T3
Température (°C)	31.10 ± 0.66 [a]	31.21 ± 0.59 [a]	30.55 ± 1.16 [a]	31.41 ± 0.77 [a]
pH	6.18 ± 0.41 [a]	6.16 ± 0.33 [a]	5.93 ± 0.63 [a]	5.95 ± 0.66 [a]
Dissolved Oxygen (mg/l)	5.36 ± 0.20 [a]	5.57 ± 0.31 [b]	5.55 ± 0.49 [c]	5.88 ± 0.54 [d]
Conductivity (µS/cm)	71.90 ± 4.01 [a]	127 ±7.03 [b]	126.72 ± 22.35 [b]	135.81 ± 24.29 [c]
NH4+ (mg/l)	0.13 ± 0.05 [a]	0.44 ± 0.27 [b]	0.42 ± 0.27 [b]	0.67 ± 0.88 [c]
N02- (mg/l)	0.007 ± 0.003 [a]	0.010 ± 0.005 [a]	0.010 ± 0.004 [a]	0.011 ± 0.006 [a]
N03- (mg/l)	4.99 ± 1.38 [a]	6.46 ± 4.19 [b]	7.97 ± 2.06 [c]	10.73 ± 3.11 [d]
P04 3- (mg/l)	1.23 ± 0.6 [a]	7.94 ± 1.67 [b]	4.70 ± 3.82 [c]	8.12 ± 4.39 [d]
Chlorophyll a (µg/l)	95.02 ± 57.83 [a]	272.95 ± 157.73 [b]	235.11 ± 106.05 [c]	336.51 ± 119.52 [d]

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

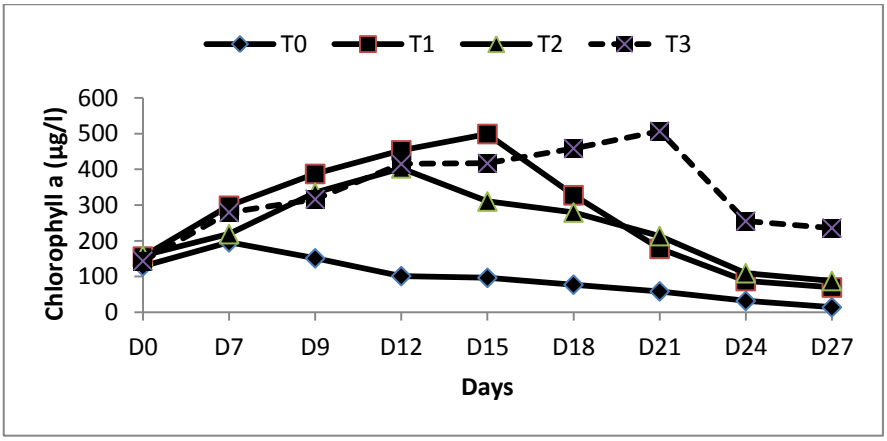


Fig.1. Evolution of chlorophyll a concentration of different treatments in function of time.

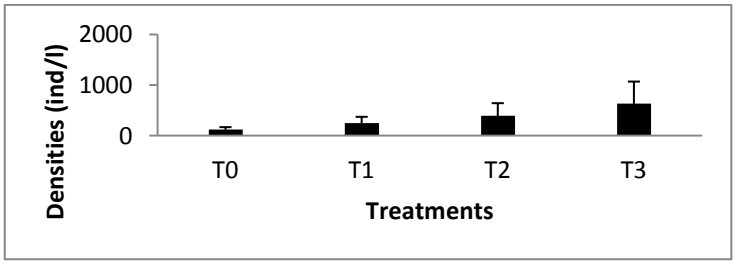


Fig.2. Zooplankton total densities by treatment.

Results presented in Figure 3 revealed the evolution of the total zooplankton densities with the time. The average zooplankton densities of fertilized medium increased progressively from D_0 to D_{12}. After D_{12}, they increased rapidly in treatment T_3 with a peak (1207 ind/l) at D_{21}, and slightly in T_2 with a peak (690 ind/l) at D_{21}. After the peaks, T_3 decreased slightly whereas T_2 fall till the end of the experiment, at D_{27} (respectively 708 ind/l and 154 ind/l). Furthermore, the density of T_1 decreased progressively from D_{15}, where it reached its peak (413 ind/l). From D_0 to D_{27}, the daily production, intrinsic increase rate and doubling time of zooplankton population for treatment T_2 were respectively 4.34 ind/l/d ; 0.05 in 24 hours and 13.11 days whereas the ones of T_3 were respectively 24.85 ind/l/d ; 0.10 in 24 hours and 6.34 days.

The analysis of figure 4 showed that the average densities of different zooplankton groups (rotifers, copepods and cladocerans) were higher in treatment T_3 medium. The culturing medium of T_3 were favored the growth of rotifers (333 ± 265 ind/l) which was followed by the copepods (274 ± 179 ind/l) whereas the medium of T_2 were dominated by copepods (227 ± 158 ind/l) and it was followed by the rotifers (150 ± 112 ind/l).

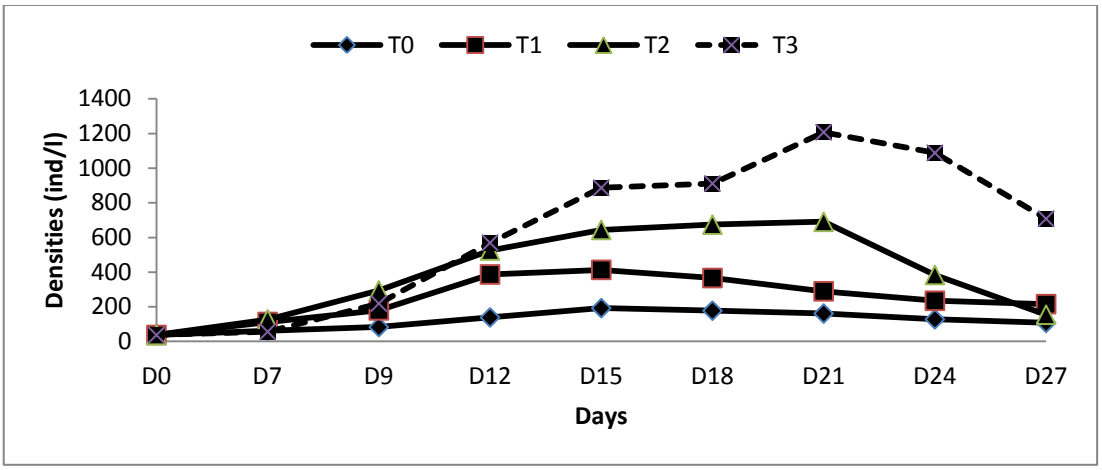
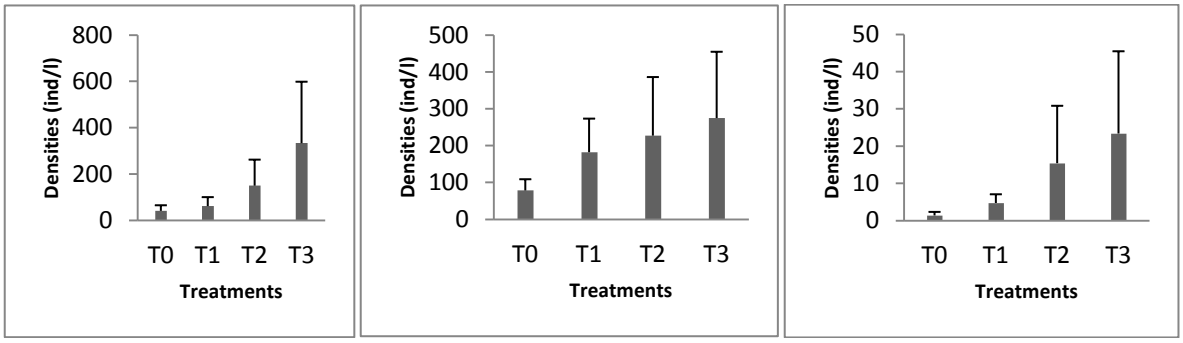


Fig.3. Evolution of total zooplankton average densities of different treatments in function of time



a- Rotifers b- Copepods c- Cladocerans

Fig.4. Different zooplankton groups' density by treatments

4. Discussion

The average temperature of buckets water was around 31.07 ± 0.8°C during the study time, this temperature is conform to that which permitted the production of high density of rotifers (28 and 32°C) obtained during the freshwater rotifers culture, Brachionus calicyflorus (Park et al., 2001). The pH average value was 6.05 ± 0.51 and slightly fluctuated. It has permitted a good zooplankton development in production medium, as this value was comprised between the one obtained by Kabir et al. (2010) for a good growth of rotifers (6-8). The conductivity and the average concentrations of NH_4^-, NO_3^-, NO_2^- and PO_4^{3-} were the highest in treatment medium T_3, as the latter were periodically fertilized during the experimentation. The periodical application of pig dung has significantly ameliorated the dissolved salts in water and it change the physico-chemical quality of the water (Adedeji et al., 2011). Results of the study were confirmed the positive effect of pig dung over the nutritious quality of water (Akodogbo et al., 2014a).

Progressive decrease in the concentration of chlorophyll *a* was observed in treatment T_1 medium from D_{15}, was due by the exhaustion of the culture medium, during the time, in nutritious salts (Agadjihouèdé et al., 2010a, 2010b; Akodogbo et al., 2014a). The fall of chlorophyll *a* concentration observed in the treatment medium T_2 from D_{12} was explained by the dilution of these culture medium by water used for the renewal. On the other hand, the treatment medium T_3, the amelioration of the chlorophyll a rate and then of the phytoplankton, was due to the periodical supply of fertilizers which liberated permanently nutritious salts required to the phytoplankton development in the production medium. The periodical fertilization has therefore a positive effect on the phytoplankton development. This confirmed that the phytoplankton development of phytoplankton depends on nutritious salts (Schlumberger and Bouretz, 2002; Akodogbo et al., 2014a). The slight fall of chlorophyll a rate towards the end of the experiment in T_3 was also due to the dilution effect.

The fall of the zooplankton density in treatment medium T_1 after D_{15} was explained by the decrease in phytoplankton density, because 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 due to the exhaustion of the fertilizers in nutritious substances required for the phytoplankton development. As in 20 days, the organic matter was completely mineralized in the water (Berard, 1993). This justified a positive correlation between the nutritious salts, phytoplankton population and the zooplankton (Lazzaro and Lacroix, 1995; Akodogbo et al., 2014a). Likewise, the fall of this density on D_{15} confirmed by the works of Akodogbo et al. (2014b) which showed that the maintain time of the pig dung optimal dose was 14 days. The zooplankton average densities in treatments T_2 and T_3 haven't fallen after D_{15} because of the renewal of their medium. We noticed a regular increase of these densities after the first three stripping (J_{21}). That renewal eliminated a part of the pollutants (metabolites) and then avoids the congestion (Orhun et al., 1991; Arimoro, 2006). It is the dilution (Fukusho, 1989b). The improvement of the total zooplanktonic density in treatment T_3 (631 ± 440 ind/l) in relation to T_2 (392 ± 253 ind/l) was due to the phytoplankton higher density in these periodical fertilized medium. The food (phytoplankton) is available for the zooplankton, therefore their good growth. That improvement of the phytoplankton density was due to the periodical fertilization, which broughted nutritious salts further to the mineralization of the pig dung (Akodogbo et al., 2014). That periodical fertilization from D_{12} increased the zooplanktonic density so as to avoid the sudden death of the population due to the lack of food (Morris and Mischke, 1999; Arimoro, 2005). The partial renewal followed by the periodical fertilization improved the zooplanktonic density which was dominated by the rotifers, live prey for the larvae of most of the fish species (Arimoro, 2006). The adoption of this production technique of zooplankton permitted to get small sized live prey in mass which could be maintained during the larval rearing period, 5-6 days for the catfish larvae (Légendre et al., 1992). It favored then the production of small size live prey for the hatcheries.

5. Conclusion

Plurispecific production of local zooplankton from pig dung could be optimized as renewed medium through the partial renewal followed by the periodical fertilization of the culture medium. The periodical fertilization favors the development of food (phytoplankton) for the zooplankton whereas the renewal avoids the sudden death of the population. The adoption of this production technique in mass of zooplankton permitted to get small sized live prey which could be maintained during the larval rearing period. It favored then the production of adequate live prey (rotifers) for the aquaculture hatcheries and the reduction of production costs.

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