



Influence of pig dung dose on zooplankton productivity in microcosm

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Abstract

An experiment on the influence of pig dung dose on the production of zooplankton was conducted in microcosm for 28 days. The experimental plan consisting of 5 treatments corresponding to the D0 (control), D10, D15, D20 and D25 g per 50 l of water was randomly distributed and in three replicates in 15 identical plastic containers of capacity. 60 l. One third (1/3) of each fertilizer dose was administered once a week as maintenance fertilizer. The zooplankton was seeded with a density of 12; 11 and 2 ind / l respectively of rotifers, copepods and cladocerans. Physico-chemical and zooplanktonic characteristics were collected every 7 days. Mean values of all physico-chemical characteristics of water remained very close to one dose and showed no significant difference ($p > 0.05$). The highest density values (6914.2 ind / l) and zooplankton biomass (1139.14 $\mu\text{gPS} / \text{l}$) were obtained with the D25 g dose, followed by 5448.2 ind / l and 626, 27 $\mu\text{gPS} / \text{l}$ respectively with the dose D20 g. The lowest density values (606.4 ind / l) and the lowest zooplankton biomass (93.91 $\mu\text{gPS} / \text{l}$) were recorded with the dose D0 g. The D25 g positively increased the nutrient content in the water as well as the production of zooplankton.

Keywords: microcosm, pig dung, zooplankton, density, biomass

Introduction

In Cameroon, almost all of the fish consumed comes only from inland and marine fisheries (FAO, 2014). However, in view of the high protein demand linked to the growing population of Cameroon, which reached 2.5% in 2010 (Brucrep, 2010), fishing alone can no longer meet fish needs on a sustainable basis because it faces a number of problems that hamper its production (FAO, 2016) ^[8]. Thus, aquaculture has become the only alternative because of its high contribution (42%) to increase the supply of permanent fish for human consumption which is around 50% (FAO, 2016) ^[8]. The development of the latter would make it possible to cover huge animal protein requirements and reduce the massive outflow of foreign currency. However, its development still faces numerous constraints, including the availability of quality fry in sufficient quantity, which is only a consequence of the mortalities associated with the larval production stage. A better production of larvae is linked to an efficient diet, especially live prey (zooplankton) because of its high protein content of about 54% (Agadjihouèdé *et al.*, 2011b and Cacot, 2007) ^[2, 5]. The use of live prey with high production potential such as rotifers, cladocerans and some copepods is less widespread but may be easier.

Research on the production of zooplankton in above-ground infrastructures (tanks, basins and aquariums) and pond focused on the use of some animal manure including poultry manure, cow dung, pig excrement and dung guinea pigs (Agadjihouèdé *et al.*, 2010, 2011, Akodogbo *et al.*, 2014, Efole *et al.*, 2017, Nana *et al.*, 2018a) ^[2, 3, 12]. These droppings are used as organic fertilizers in fish farming to promote the development of phytoplankton and zooplankton ricrochet in order to improve fish production (Nana *et al.*, 2018b) ^[13]. The optimal dose of use of poultry droppings in an above-ground

environment is already known (Agadjihouèdé *et al.*, 2010, 2011) ^[2]. However, that of pig manure, fertilizer available, accessible and cheaply by the number of livestock structure present in the highlands of western Cameroon (Ndébi *et al.*, 2009) ^[14] is not yet determined. Thus, the search for the optimal dose of pig manure for a better production of zooplankton for aquaculture purposes without risk of eutrophication of the medium remains essential. The purpose of this study is to contribute to a better production of zooplankton through animal dejection. More specifically, the aim is to evaluate the effect of the pig manure dose on the physicochemical characteristics of water, the density and the biomass of zooplankton.

Material and methods

Period and zone of the study

The trial was conducted between April 24 to May 28, 2017 at the Application and Research Farm and the Research Unit of Ichthyology and Applied Hydrobiology of the University of Dschang. Located in the agro-ecological zone of the western highlands of Cameroon, Department of Menoua and Dschang district (5 ° 17' - 5 ° 44 'L N and 10 ° 04' - 10 ° 22 'L E). The average altitude is 1420 m and the average temperature (ambient) is 20 ° C. The annual rainfall varies between 1800 mm, with a rainy season (mid-March to mid-November) and a dry season (mid-November to mid-March).

Experimental plan

The experimental set-up consists of 15 plastic basins (circular shape, volume 60 liters, depth 0.30 m) arranged in a random and triplicate manner in a greenhouse (1.20 m high, 2 m wide and 5 m length). Each basin received 40 liters of borehole water (not containing zooplankton), followed by a initial

fertilizer application of 10, 15, 20 and 25 g / 50 l at the start of the test and one-third (1 / 3) of these different doses every week as maintenance fertilization for the maintenance of zooplankton productivity. Pig dung (solid matter + urine) was collected from a pigsty fed with standard feed. Before use, 25 g sample was taken and analyzed for total dry matter, nitrogen and total phosphate (AOAC, 1990) concentration of 16.5, respectively; 0.92 and 0.63%.

Zooplankton

Two days later, each fertilized basin received phytoplankton from a 10-liter sample of water from a fertilized pond filtered with a plankton net. The mixture was left for 72 hours, sufficient time to allow its development (Guiral *et al.*, 1994) before being seeded into zooplankton. Zooplankton was collected in a pond using a 50 µm mesh zooplankton net. For a qualitative and quantitative inventory of the different zooplankton groups that were used to inoculate the production medium, a 5L sample of water was collected, filtered and a 25 mL sub sample of 5% formalin the proportions of 25% formalin and 75% sample, then stored in a 50 ml bottle. Each basin was seeded at a density of 25 individuals including 12 ind / l of rotifers, 11 ind / l of copepods and 2 ind / l of cladocerans.

Monitoring the production of zooplankton

Zooplankton was sampled every 7 days after seeding for 28 days (D28). After homogenization of each basin, a sample of 5 l of water was collected and filtered using a plankton net of 50 microns mesh opening. Subsequently, a 25 ml subsample of zooplankton concentrate was retained, fixed at 5% formalin, labeled and stored in 30 ml plastic bottles for quantitative and qualitative laboratory analyzes. Sampling is done every morning at the same time (7-9 hours) and after this harvest the filtered water is returned to the basin.

Determination of the physicochemical characteristics of water

The physicochemical characteristics of the water of each treatment were measured at the beginning of the test then every week. The water temperature (° C), dissolved oxygen (ml / l), pH (IU), conductivity (ml) and depth (cm) were measured in situ respectively with the aid of an Oxy-thermometer, a pH meter, a conductivity meter and a ruler. One liter of water from each basin was taken and stored in one-liter double-capped polyethylene bottles for the determination of nitrate, nitrite, ammonium and phosphate respectively by the methods of nitrate III, nitrate III, Nessler-380 and phosphor by molecular absorption spectrophotometry (HACH DR / 2010).

Measurement of zooplankton characteristics

In each basin, zooplankton density and biomass were determined after binocular identification and counting (Motic at Objective 4X). After homogenization of the sample, 10 ml were taken using a calibrated pipette and introduced into a Petri dish 90 mm in diameter, squared in squares of 5 mm side. The count of at least 100 individuals per sample has been

completed and when this number is not reached, counting is done until the sample is exhausted.

Density

The density of zooplankton was calculated from the following Cacot (2007) formula:

$D = (n / v1) \times (v2 / v3)$ where n = number of individuals counted; v1 = volume of sub sample taken; v2 = volume of the concentrated sample and v3 = total volume of filtered water.

Biomass

Biomass was calculated according to the individual weights of the different zooplankton groups proposed by Gras and Saint-Jean (1981); Legendre *et al.* (1987) namely 0.07 µg / ind for rotifers, 0.08 µg / ind for copepod nauplii, 0.47 µg / ind for copepodites and adults of copepods; then finally 3.5; 2.7; 0.2 µg / ind (dry weight) for adult females, juveniles and neonates of *Moina micrura* (dominant species of cladocerans) respectively.

$B = D \times P$ (µgPS / l) with: D = density (ind / l), P = weight (µgPS) and PS = dry weight

Statistical analyzes

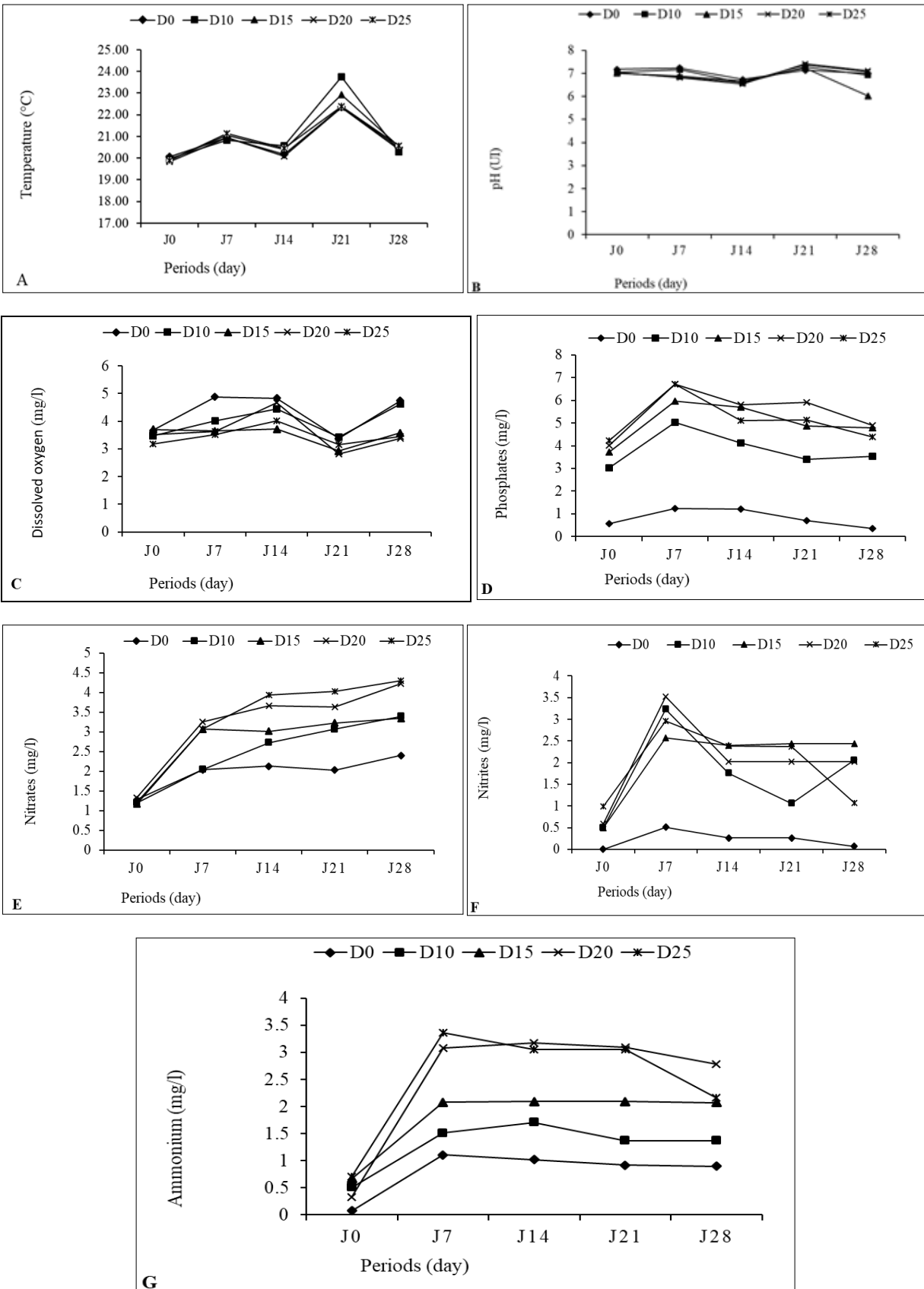
The collected data were subjected to one-way analysis of variance (ANOVA 1), when the treatment effects were significant the Duncan test was used to separate the 5% threshold means. Correlation tests were used to determine the relationship between water characteristics and zooplankton density. All analyzes were performed using SPSS software version 20.0.

Results

Physico-chemical characteristics of water

The evolution of the physicochemical characteristics of water as a function of the dose of pig manure illustrated in figure 1A to G was comparable between all the treatments. The temperature values changed little with a fluctuation range of ± 1.52 ° C around 21 ° C with the lowest values recorded at the beginning of the test and the highest values on the 21st day (Figure 1A). The pH values fluctuated very little by ± 0.46 IU around 6.77 IU and remained very close to one dose during the test (Figure 1B). In general, in all doses, dissolved oxygen levels have varied and are highest in the control treatment (D0) (Figure 1C).

The levels of phosphate ions followed the same progression in all doses during the test with the peaks recorded on the 14th day, and then decreased until the end of the test (Figure 1D). Nevertheless, the highest values (p <0.05) were recorded in doses D20 g. As for nitrates, the levels increased with the pig dung dose. Thus, the lowest values being recorded with the control treatment (D0 g) and the strongest with D25 g (Figure 1E). The nitrite values oscillated and the highest values were observed at the 7th day with the D20 dose and the lowest values with the D0 g treatment (Figure. 1F). Ammonium levels also increased with the pig dung dose. Thus the lowest values recorded with the dose D0g and the highest values with the dose D25g (Figure 1G).



A = temperature; B = depth; C = pH; D = dissolved oxygen; E = phosphates, F = nitrates; G = nitrites and H = Ammonium. 0, 7, 14, 21 and 28 = day of data collection, D0, D10, D15, D20 and D25 = respective fertilization dose at 0, 10, 15, 20 and 25 g / 50 l

Fig 1: Evolution of the physicochemical characteristics of water according to the dose of pig dung.

Zooplanktonic characteristics

Density of zooplanktonic groups

The density of zooplankton as a function of the pig slurry dose (Figure 2) shows that whatever the treatment, the zooplankton density follows a regular evolution until it reaches the peak on the 21st day. Thus, the highest values were recorded with the dose D25 g and the lowest with the dose D0 g.

When considering the groups (Figure 3), it appears that whatever the group, the density increased significantly ($p < 0.05$) with the dose of pig manure. However, the highest density value (6914.2 ind / l) was obtained with the dose D25 g, followed by D20 g (5448.2 ind / l) and the lowest with D0 g (606.4 ind / l). At the peak, the highest density is obtained with the dose D25 g (4934.67 ± 4349.34 ind / l) corresponding to a composition of 38% of rotifers, 2% of cladocerans and 60% of copepods. Monitoring of D20 g (3437 ± 2968.82 ind / l) of which 46% of rotifers, 1% of cladocerans and 53% of copepods. The lowest density is obtained with the D0 g dose (537.66 ± 466.80) of which 56% of rotifers, 1% of cladocerans and 43% of copepods.

From the 21st day until the end of the experiment, the density of zooplankton dropped gradually in all environments. The

copepods recorded the highest ($p < 0.05$) density significantly (37.82 ind / l) with the D25 g dose compared to the D0 g dose (2.48 ind / l) (Table 1). The density (30.17 ind / l) of the rotifers of the dose D25 was significantly ($p < 0.05$) higher compared to the doses D0 and D10 g respectively with the values 3.53 ind / l and 5.79 ind / l. The density of cladocerans (1.16 ind / l) was significantly ($p < 0.05$) highest with the dose D25 g compared to other doses

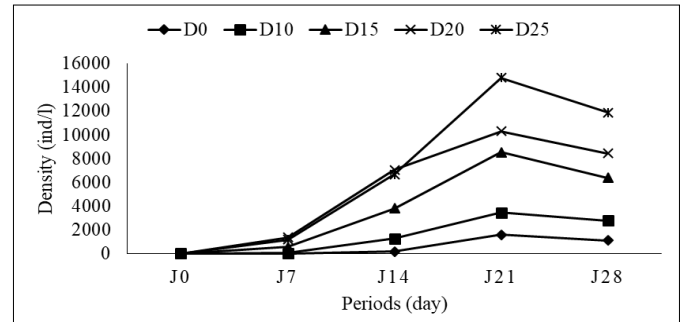
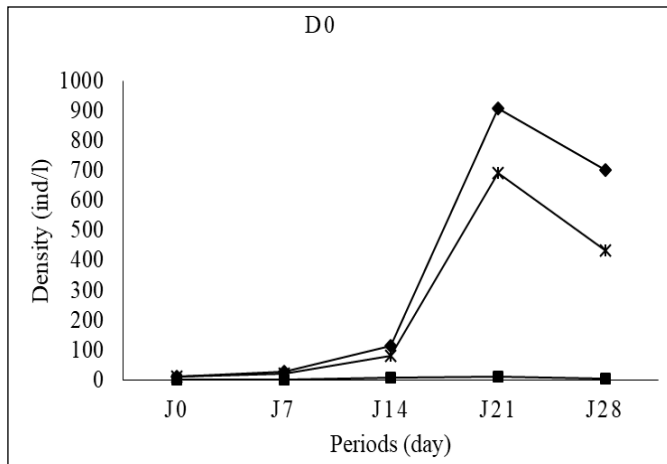
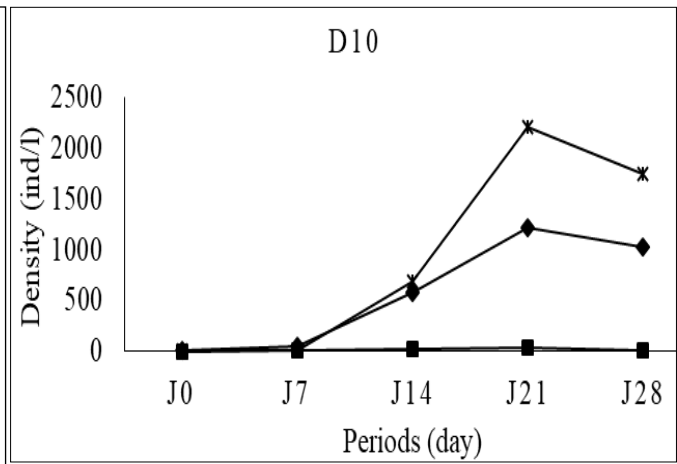


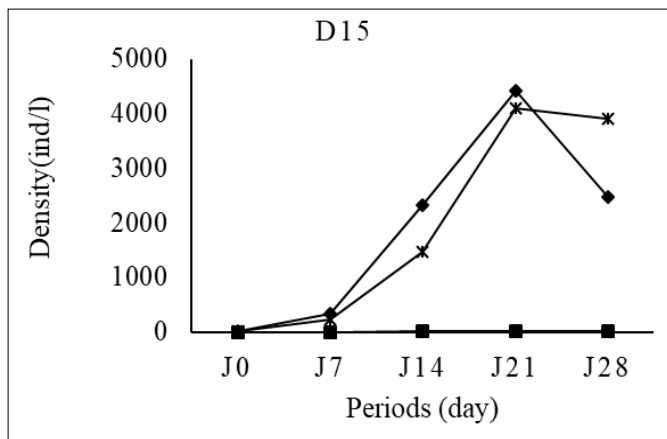
Fig 2: evolution of zooplankton densities as a function of pig dung dose.



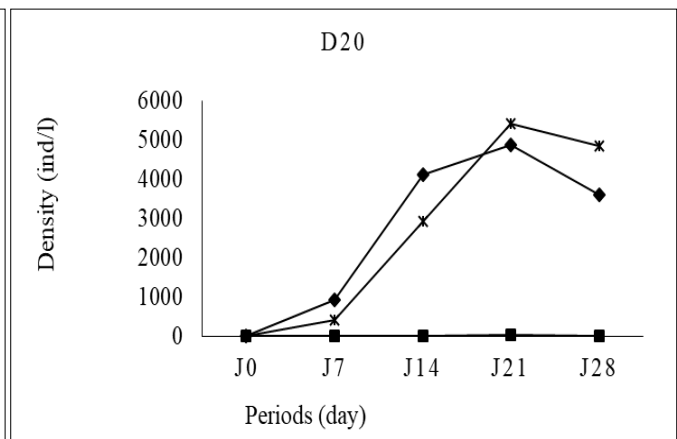
A



B



C



D

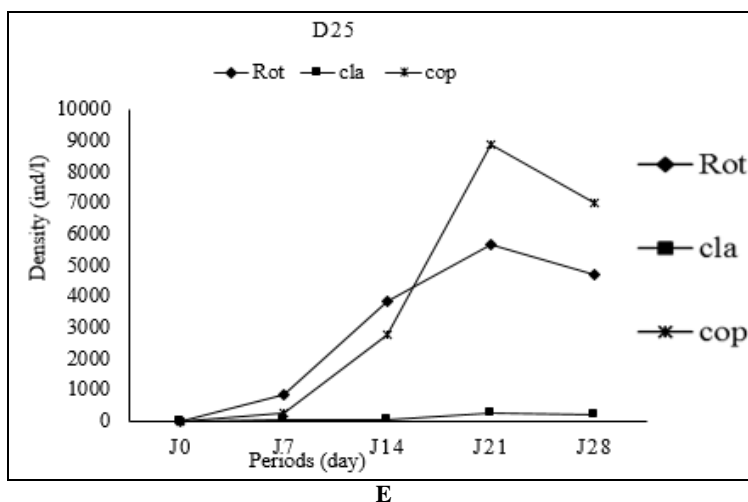


Fig 3: Evolution of the density of zooplankton groups as a function of the dose of pig dung.

Table 1: Zooplankton group density as a function of the dose of pig dung

Zooplankton group	Doses (g)				
	D0	D10	D15	D20	D25
Rotifers (ind/l)	353,00±420,76 ^a	579,20±551,31 ^a	1913,00±1786,68 ^{ab}	2715,00±2117,78 ^{ab}	3017,00±2458,99 ^b
Cladocerans (ind/l)	5,60±4,62 ^a	12,20±13,59 ^a	13,00±10,68 ^a	11,80±8,82 ^a	115,60±110,79 ^b
Copepods (ind/l)	247,80±303,30 ^a	934,80±1007,32 ^{ab}	1945,20±1961,41 ^{ab}	2721,40±2472,44 ^{ab}	3781,60±3996,93 ^b

a, b and c: averages with identical letters on the same lines show no significant difference ($p > 0.05$). D0, D10, D15, D20 and D25: ponds fertilized respectively at 0, 10, 15, 20 and 25 g / 50 l of pig dung

Biomass of zooplankton groups

The biomass of the zooplankton groups as a function of the slurry dose (Figure 5) shows, just as the density, the evolution of the biomass peaked on the 21st day. Thus, the highest values were recorded with the dose D25 g and the lowest with the control treatment (D0 g). During the peak, the highest biomass is obtained with the dose D25 g ($1139.14 \pm 1132.53 \mu\text{gPS} / \text{l}$) corresponding to a composition of 12% of rotifers, 17% of cladocerans and 71% of copepods. Monitoring of D20 g ($626.27 \pm 760.94 \mu\text{gPS} / \text{l}$) including 18% rotifers, 3% cladocerans and 79% copepods. The lowest biomass is obtained with the D0g dose ($93.91 \pm 85.59 \mu\text{gPS} / \text{l}$) of which 22% rotifers, 10% cladocerans and 68% copepods.

For zooplankton groups, copepod biomass was significantly ($p < 0.05$) highest ($1777.35 \mu\text{g PS} / \text{l}$) with D25 g compared to biomass ($37.82 \mu\text{gPS} / \text{l}$) of the dose D0 g (Table 2). On the other hand, the rotifer biomass ($211.19 \mu\text{gPS} / \text{l}$) recorded with the D25 treatment was significantly higher compared to

The D0 and D10g treatments respectively with the values $30.17 \mu\text{gPS} / \text{l}$ and $40.54 \mu\text{gPS} / \text{l}$. otherwise comparable. Cladoceran biomass ($23.12 \mu\text{gPS} / \text{l}$) was highest ($p < 0.05$) with D25 g dose compared to other doses

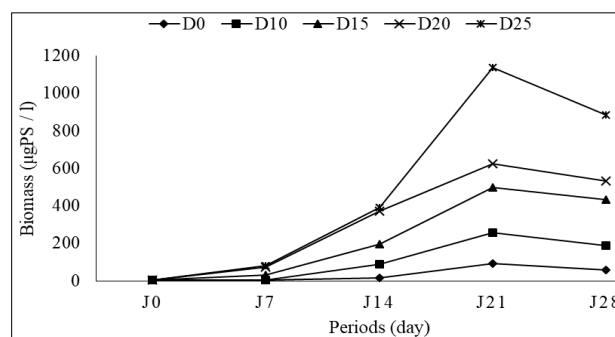
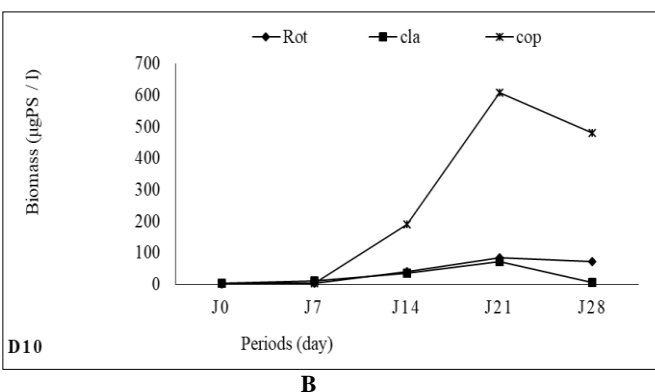
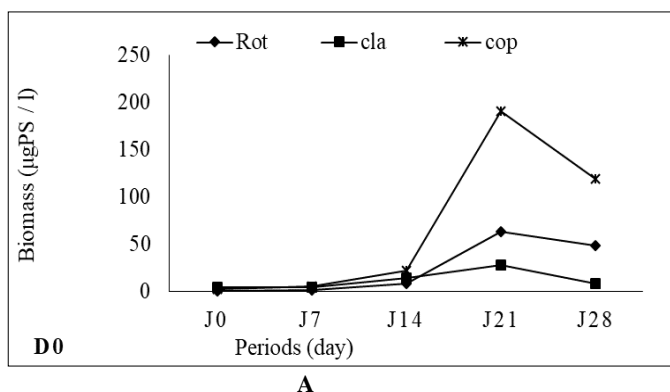


Fig 4: Evolution of zooplankton biomass as a function of the dose of pig dung.



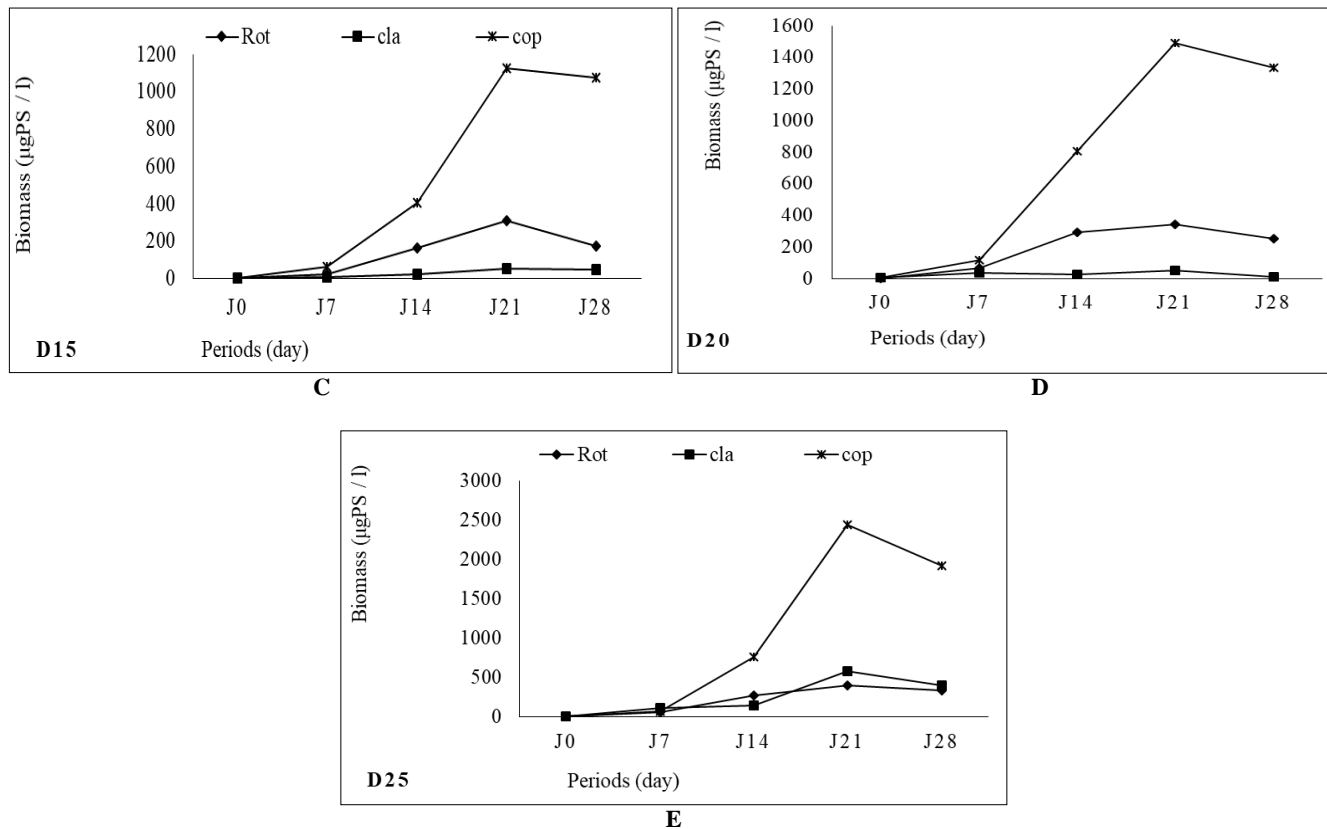


Fig 5: evolution of the biomass of different groups of zooplankton as a function of the dose of pig dung.

Table 2: Biomass of zooplankton groups by pig dung dose

Zooplankton group	Doses (g)				
	D0	D10	D15	D20	D25
Rotifers (µgPS/l)	24,71±29,45 ^a	40,54±38,59 ^a	133,91±125,06 ^{ab}	190,05±148,24 ^{ab}	221,20±172,10 ^b
Cladocerans (µgPS/l)	11,93±9,83 ^a	25,99±28,95 ^a	27,69±22,59 ^a	25,13±18,77 ^a	246,20±23 ^b
Copepods (µgPS/l)	68,15±83,41 ^a	257,07±277,01 ^{ab}	534,93±539,38 ^{ab}	748,38±679,92 ^{ab}	1039,94±1099,15 ^b

a, b and c: averages with identical letters on the same lines show no significant difference ($p > 0.05$). D0, D10, D15, D20 and D25: ponds fertilized respectively at 0, 10, 15, 20 and 25 g / 50 l of pig manure

Discussion

The fertilizing effect of pig manure has favored the development of the highest algal biomass D25, D20 and D15 g for good primary production and therefore provides better nutritional conditions for zooplankton. The highest density and biomass values were obtained in hog manure ponds. High zooplankton densities were obtained with the fertilized basins and the lowest densities obtained with the control (unfertilized). The increasing zooplankton density values with fertilization level (nitrogen and phosphorus content) could be explained by the physico-chemical characteristics (pH, nitrite, nitrate and phosphorus) of the medium which had average values required for a good response to fertilization. This would be the consequence of the algal richness of this medium which induces the multiplication of zooplankton according to Seyer (2002) [17]. The dose D25 g gave a better production of zooplankton. The average production obtained with this dose makes it possible to multiply by 1.3; 1.8; 4.5 and 11.4 times respectively with the average yields of D20, D15, D10 and D0g in terms of density. In terms of biomass, this dose makes it possible to multiply by 1.5; 2.2; 4.6 and 14.3 times

respectively with the average productions of D20, D15, D10 and D0 g. This could be explained by the change due to the phytoplankton richness of the medium, which increases proportionally with the weekly dose of pig manure. The same is true of the density and biomass values of the different zooplankton groups. In terms of density, rotifers develop rapidly one week after fertilization compared to cladocerans and copepods, which are abundant two weeks later in all production environments. This could be explained by the fact that rotifers with a varied mode of nutrition are capable of filtering suspended organic matter in order to grow rapidly and reproduce (ACF 2010) [1]. In addition, the slow increase in copepod density confirms that nauplii only become adults after 6 successive metaphases, Soulat (2011). The rapid decline of rotifers from day 21 in all production environments is due to the increase in the density of predatory copepods. The highest biomass value was obtained with copepods in all doses. This would be related to the difference in weight between zooplankton groups (0.07 mg / ind for rotifers, 0.08 mg / ind for nauplius copepods and 0.47 mg / ind for adult copepod mixtures) reported by Dabbadie (1996) [6]. The

highest intrinsic rate of zooplankton increment was recorded with fertilized basins. The highest value obtained with the dose D25 g would be linked to the richness of the medium in nitrite, nitrate and phosphorus which favored the multiplication of rotifers and copepods. According to Shep (1994) [18], the intrinsic rate of increase in multispecies breeding depends on that of the dominant species group. The values of the relative abundance of the highest zooplankton groups were recorded with fertilized basins and could be explained by the phytoplankton richness of this medium, which is only a consequence of the presence of nitrogen and phosphorus content. In pig dung.

Conclusion

The effect of the pig slurry dose on microcosmal zooplankton production has shown that water fertilization with pig manure improves the physicochemical characteristics of the water. This fertilization is also responsible for a large zooplankton production composed of rotifers, copepods and cladocerans. The D25, D20 and D15 g doses gave good production in terms of density and biomass, of the different zooplanktonic groups. Thus, the highest values were obtained with the highest dose (25g).

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