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Effects of the Level and Frequency of Fertilization with hen Droppings on Zooplanktonic Density and Growth Performance of Common Carp Post-Larvae (*Cyprinus Carpio*)

Djikengoue Kameni Patricia Linda[1,2], Mambe Tameghe Flora[1], Kpoumie Nsangou Amidou[2,*], Tonfack Achile Peguy[1,2]

[1]Aquaculture and fishing laboratory, Agricultural Research Institute for Development (IRAD), Specialized Station of Foumban-Cameroon

[2]University of Dschang, Department of Animal Science, Laboratory of Ichthyology and Applied Hydrobiology

Abstract

Zooplankton production and growth performance of post-larvae of common carp according to the level and frequency of fertilization with chicken droppings were studied between May and October 2017 at the IRAD pisciculture station in Foumban. To this end, two doses of hen droppings, namely 450 g/m^3 (D450) and 600 g/m^3 (D600), were each applied at two application frequencies (weekly (F2) and bimonthly (F1)). Thus, 1200 post-carp larvae were distributed in 12 identical concrete tanks (1.2m x 0.75m x 1m) each filled with 400 liters of water. The 2 doses applied at 2 frequencies were applied randomly in the 12 tanks in a complete random device comprising 3 treatments and 2 repetitions. Six days after fertilization, each tank was sown with zooplankton at a density of 7 individuals per liter (ind/l). The loading was carried out 12 days after fertilization at a density of 100 ind/m^2. The results show that regardless of the dose and the frequency of droppings applied, the production of zooplankton was optimal 10 to 12 days after fertilization. Considering the growth performance, the tanks receiving the 600 g/m^3 dose every 2 weeks presented the highest significant values ($p < 0.05$). On the other hand, the survival rate (36% on average) was not significantly influenced ($p > 0.05$) by the dose and the frequency of fertilization. The 600 g/m^3 dose applied every two weeks can be recommended for the rearing of post-larvae common carp.

Corresponding author: Kpoumie Nsangou Amidou, University of Dschang, Department of Animal Science, Laboratory of Ichthyology and Applied Hydrobiology

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Introduction

In Africa, nutritional deficiencies in proteins of animal origin are sources of metabolic diseases and from which a large part of its population suffers [1, 2]. Of all the sources of protein, fish represents a reserve of 16.6% in animal proteins and 6.5% of the total proteins consumed in the world [3]. In Sub-Saharan Africa and more specifically in Cameroon, 46% of the 20g of animal protein consumed per day per inhabitant is covered by fish. Most of this fish comes from fisheries and imports and only 1% from aquaculture [4]. However, the decline in peach stocks in recent years due to the overfishing, destruction of wetlands and climate change are render aquaculture the only alternative to fight the unsatisfied fish needs of the populations [5, 6]. Now this sector has faced five major constraints for 50 years, the main one being the lack of quality fry [7]. This scarcity of fry is linked to the low larval survival rates in hatcheries, which would be due to several reasons, the main one being the lack of control over the qualitative and quantitative production of adapted live prey such as zooplankton [8]. To this end, several studies like those of [9, 10] have shown that the use of agricultural waste and mainly chicken droppings allows better production of these zooplankton. The work of [10] and [11] suggest 600 g/m^3 of chicken droppings as the best level of fertilization with a peak production obtained 10 to 12 days after fertilization. According to these authors, the evolution of the density of zooplankton indicates a sudden drop in the density of these organisms just after the peak of production. Thus, the real challenges lie in the constant maintenance of this production in order to ensure optimal growth of the post-larvae. However, to our knowledge, very few studies are available on the frequency of fertilization and its impact on post-larval growth. The general objective of this work is to contribute to better knowledge of the production of quality fry through better control of zooplankton production factors. More specifically, the aim was to assess the effect of the level and frequency of fertilization with chicken droppings on the density of zooplankton, on the survival and growth performance of common carp post-larvae (*Cyprinus carpio*) in concrete tanks.

Material and Methods

Study Area

The study took place from March to May 2018 at the IRAD pisciculture Station at Foumban, more precisely at the Koupa-matapit fish farm (5º21 'to 5°58' LN: 10º17 'to 11º02' to 10°48.82` LE) and an altitude of 1147m in the Western Region of Cameroon. The climate is of the Sudano-Guinean-type and includes a rainy season (March-October) and a Dry season (November-February). The average values of the temperature and the rainfall recorded annually respectively are 22°C and 1800 mm [12].

Preparation of Concrete Tanks and Fertilizer

The test was carried out in 12 identical concrete tanks, 1.2 m long, 0.75 m wide and 1 m high each. These tanks were covered with plastic material in order to keep the temperature of the ambient environment constant. Two weeks before the start of the test, the tanks were washed and disinfected with bleach and then left to dry for 3 days. In addition, each tank received three days after draining a volume of 400 liters of drilling water and 21 grams of quicklime.

The droppings of laying hens (analyzed composition: 19.24% of crude cellulose; 0.87% of nitrogen and 0.61% of phosphorus) were used.

Biological Material

A concentrated sample of zooplankton was collected between 6 and 7 a.m in a Tilapia pond at the Station using a 40 μm mesh trawl pulled from one side of the pond to the other. This zooplankton sample was homogeneously distributed in 13 test tubes of 25 ml each. 1200 post-larvae of *Cyprinus carpio* were caught in the nursery tanks of the Koupa-Matapit Station just after absorption of the yolk sac. These post-larvae came from a semi-artificial reproduction according to the model described by [12].

Conduct of the Test

One week after liming, each of the tanks was fertilized randomly and in three repetitions at one of the following doses of droppings: D450-F1 (fertilization at 450 g/m^3 applied every 2 two weeks), D450-F2 (fertilization at 450 g/m^3 at weekly frequency), D600-F1 (fertilization at 600 g/m^3 at biweekly frequency) and D600-F2 (fertilization 450 g/m^3 weekly). Three days after this fertilization the tanks were sown with phytoplankton (food of the future zooplankton). For this purpose, 100 liters of water from a tilapia storage pond were withdrawn, filtered to 50µm of meshes in order to let only the phytoplankton pass, then added in each tank. Three days later, a sample of zooplankton was collected between 6 and 7 a.m in a Tilapia pond from the same Station. As described above, this zooplankton sample was concentrated and distributed homogeneously in 13 test tubes of 25 ml each. The 12 tanks received each the contents of one tube. The content of the 13th tube was fixed with 5% formalin and then immediately sent to the IRAD aquaculture laboratory at Foumban for a qualitative and quantitative inventory of the zooplankton under the MOTIC type optical microscope at the 10X objective [9]. It emerged that the zooplankton was inseminated in the tanks at a density of 7 ind/l (that is 1; 2 and 4 ind/l of rotifers, cladocerans and copepods, respectively). 12 days after fertilization, each 1 m^2 concrete tank received 100 *Cyprinus carpio* post larvae aging 5 days.

Data Collection

Zooplankton production was assessed every three days during the 30 days of the trial following the protocol described by several authors [10, 11]. For this, every three days, between 6 and 8 a.m., 20 liters of water were withdrawn from each tank using a one-liter container (therefore 15 sampling points on the edges and 05 points in the middle of the tank). This water was filtered through a 50µm zooplankton screen to retain the zooplankton which was then stored in a 25 ml test tube then fixed with 5% formalin and sent to the IRAD aquaculture laboratory at Foumban for observation according to the protocol described by [11]. To follow the evolution of the zootechnical performances of common carp larvae, for 30 days, control fisheries were carried out every two weeks, within at least 10% of the individuals in each tank were sampled and the lengths (total and standard) were measured using a millimeter ichtyometer. The weight was measured using a 0.05g precision electric balance.

The physico-chemical characteristics of the water were measured at the start of the test and then weekly between 6 and 8 a.m such as the pH, the dissolved oxygen, the temperature, the conductivity, the transparency and the depth of the water have been relieved in situ. On the same dates, a water sample was collected for the determination of nutrient salts, namely: nitrates, nitrites, ammoniacal nitrogen and phosphates. All these physico-chemical characteristics are within the acceptable range for zooplanktonic production the best growth of *Cyprinus carpio.*

Studied Parameters

At the end of trial, the live weight, the total and standard lengths as well as the number of zooplankton made it feasible to evaluate the following growth characteristics:

$$D = \frac{n}{v_1} \times \frac{v_2}{v_3}$$

Zooplankton density (D): With: n = number of individuals counted; v_1 = volume of the subsample taken; v_2 = volume of the concentrated sample; v_3 = total volume of filtered water); Weight gain (WG) = final weight - initial weight; Average daily gain (ADG) = (final weight - initial weight) / (time (day)); Specific growth rate (SGR) = ((ln final weight - ln initial weight)*100) / (day time); Condition factor K = 100*(Weight / (Total length)^3) and Survival rate = (Number of initial fish - final number of fish)*100 / (Number of initial fish);

Statistical Analyzes

The data were submitted to the one-way analysis of variance (ANOVA 1). When the effect of the dose associated with frequency was significant, the Duncan test was used to separate the means clustering at 5% threshold. The regressions tests were done using Excel 2007. All analyzes were Performed using the SPSS software version 21.0.

Results

Effect of the Level and Frequency of Fertilization with hen Droppings on the Evolution of zooplankton Groups

The evolution over time of the average density of zooplankton according to the dose of chicken droppings and the frequency of fertilization in carp nursery tanks is illustrated in Figure 1. It shows that independently of the dose of droppings and the frequency of fertilization the evolution of the density of zooplankton follows the same profile, trend and pace. Thus, we observe rapid growth from the insemination of

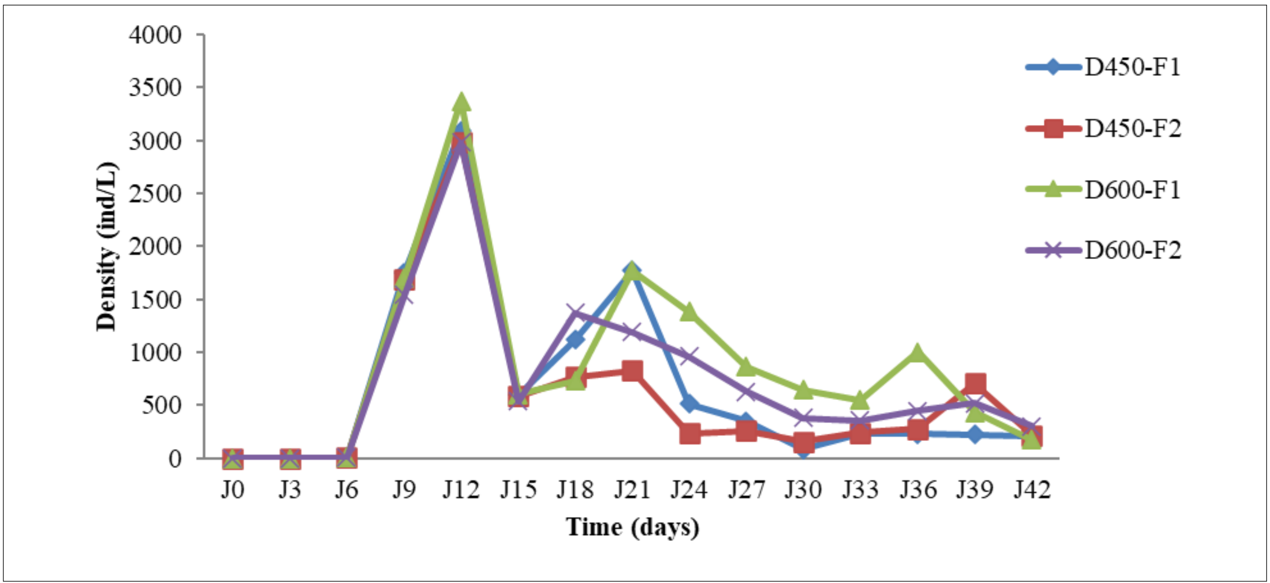

Figure 1. Evolution of the average density (ind/l) of zooplankton according to the dose and the frequency of fertilization with hen droppings in nursery

F2: weekly fertilization, F1: fortnightly fertilization, D450, D600: tanks fertilized respectively at 450 and 600 g/l of hen droppings.

zooplankton between day 6 and day 12, followed by an abrupt drop of the density of zooplankton three days after fish stocking (15th day), then occurs a slow and regular growth phase.

Effects of the Level and Frequency of Fertilization with Chicken Droppings on the Density of Zooplankton Production

The total density of the different groups of zooplankton is summarized in Table 1. It appears that with the exception of the copepods whose highest significant values were obtained with the dose 600 g/m^3 applied every 2 weeks, all the other groups presented the highest density values with the dose 600 g/m^3 applied each week

Effects of Level and Frequency of Fertilization with hen Droppings on the Growth and Survival Characteristics of Cyprinus Carpio Post-Larvae

The influence of the level and frequency of fertilization with hen droppings on the survival rate of *Cyprinus carpio* post-larvae is illustrated in Figure 2. It follows that the survival rate was not significantly influenced ($p < 0.05$) by the level and frequency of fertilization.

Effects of the Dose of Chicken Droppings and the Frequency of Fertilization on the Growth Parameters and Survival of the Cyprinus Carpio Post-Larvae

The influence of the dose of hen droppings and the frequency of fertilization on the growth and survival of the *Cyprinus carpio* post-larvae is presented in Table 2. It appears that the tanks having received 600 g/m^3 each 2 weeks presented the highest significant values of bodyweight, weight gain, average daily gain and condition factor K.

Correlations Between Growth Characteristics of Common Carp Post-Larvae and the Density of Groups of Zooplankton

It appears from table 3 presenting the correlations between density of zooplankton groups and growth characteristics that the zooplankton density of the medium did not significantly influence the zootechnical characteristics of common carp post-larvae, except for copepods and cladocerans which were significantly negatively and very strongly correlated ($P < 0.01$) with the specific growth rate of post-larvae from tanks receiving 450 g/m^3 and 600 g/m^3 every two months and which were significantly positively and

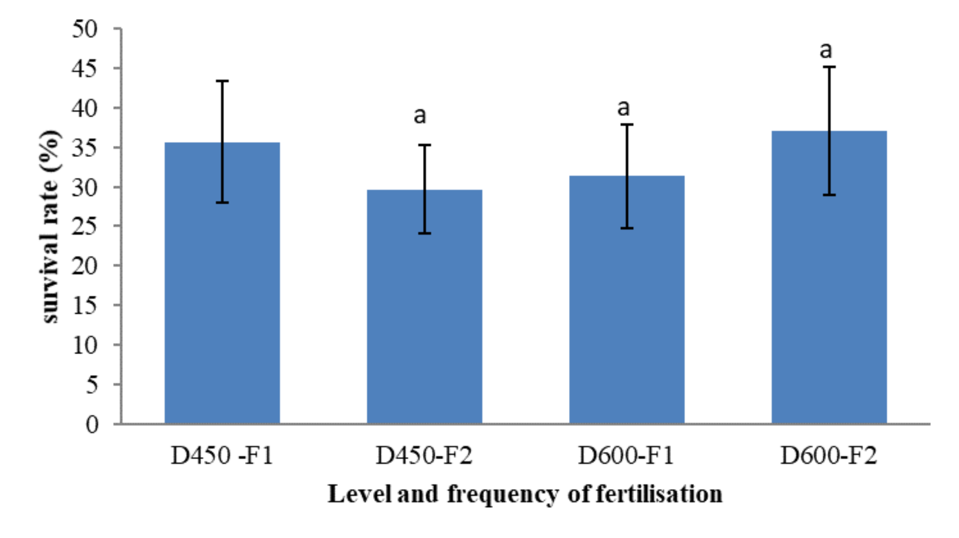

Figure 2. Survival rate according to the level and frequency of fertilization with hen droppings

F2: weekly fertilization; F1: fortnightly fertilization; D450; D600: tanks fertilized respectively at 450 and 600 g/l of hen droppings. ** Significant correlation (P <0.01); * Significant correlation (P <0.05).

Table 1. Density of zooplankton groups as a function of the level of chicken droppings and the frequency of fertilization

Zooplanktonics groups	Species	Fertilization level and frequency			
		D450-F1	D450-F2	D600-F1	D600-F2
Rotifers	*B.calyciflorus*	47.93 ± 2.99a	46.81 ± 7.17a	146.34 ± 1.33b	106.74 ± 0.20c
	Keratella sp.	28.58 ± 4.39a	30.39 ± 3.29a	44.49 ± 1.20b	42.05 ± 0.15b
	A. fissa	19.83 ± 1.93a	19.25 ± 1.99a	31.40 ± 1.32b	30.16 ± 0.25b
	Total Rotifers	96.35 ± 8.19a	96.45 ± 9.19a	222.23 ± 1.19b	178.94 ± 0.20c
Cladocerans	*Daphnia sp.*	236.25 ± 28.73a	406.3 ± 50.71b	282.00 ± 38.60c	239.8 ± 27.28a
Copepods	Nauplius	5.33 ± 0.20a	4.64 ± 0.37a	9.09 ± 0.56b	12.04 ± 1.58b
	Cyclops sp.	12.66 ± 1.20a	14.88 ± 0.68b	15.67 ± 1.71b	42.43 ± 0.60c
	Total copepod	17.99 ± 1.15a	19.53 ± 1.01a	24.76 ± 2.16b	54.48 ± 1.63c
Total	zooplankton	350.59 ± 30.4a	522.28 ± 54.8d	528, 99 ± 47.7b	473.22 ± 33.4c

a, b, c the means bearing identical letters for the same characteristic are not significantly (P> 0.05) different. F2: weekly fertilization; F1: fortnightly fertilization; D450; D600: tanks fertilized respectively at 450 and 600 g/l of hen droppings.

Table 2. Growth characteristics and survival of *Cyprinus carpio* post larvae according to the level of hen droppings and the frequency of fertilization

Growth characteristics	Levels of hen droppings			
	D450-F1	D450-F2	D600-F1	D600-F2
Body weight (mg)	510±18.8ab	537± 23.3b	620±23.8b	420±15.4a
Total length (mm)	33.30±4.66b	33.63±4.26b	36.20±4.41c	29.10±4.85a
Weight gain (mg)	399 ± 16.5b	360 ± 22b	431 ± 23b	180 ± 15a
ADG (mg / d)	27 ± 1.1b	24 ± 1.5b	29 ± 1.6b	12 ± 1.2a
K factor	0.99±0.43ab	1.17±0.65ab	1.24±0.51b	0.93± 0.42a
SGR (%)	10.85±1.83c	7.44± 2.36b	8.07± 2.50b	3,63± 2,18a

a, b, c the means bearing identical letters for the same characteristic are not significantly (P> 0.05) different. F2: weekly fertilization; F1: fortnightly fertilization; D450; D600: tanks fertilized respectively at 450 and 600 g/l of hen droppings. ADG: average daily gain; SGR: specific growth rate.

Table 3. Correlation between growth characteristics of common carp post larvae and density of zooplankton groups

Growth characteristics of post-larvae	Treatments	Zooplankton groups			
		Rotifers	Copepods	Cladocerans (*Daphnia sp.*)	Zooplankton
ADG	D450 - F1	-0.329	0.577	0.539	0.164
	D450 - F2	0.898	-0.994	0.354	0.864
	D600 - F1	0.208	-0.972	-0.978	0.110
	D600 - F2	-0.591	-0.832	-0.763	-0.692
K	D450 - F1	-0.490	0.712	0.382	-0.012
	D450 - F2	0.896	-0.994	0.359	0.861
	D600 - F1	-0.901	-0.456	-0.029	-0.940
	D600 - F2	-0.582	-0.838	-0.770	-0.683
SGR	D450 - F1	0.958	-1.000 **	0.366	0.703
	D450 - F2	0.939	-1.000 **	0.257	0.911
	D600 - F1	0.436	-0.888	-0.999 *	0.345
	D600 - F2	-0.576	-0.842	-0.775	-0.678
Survival	D450 - F1	-0.960	0.844	-0.815	-0.977
	D450 - F2	0.650	-0.352	-0.817	0.704
	D600 - F1	0.055	1.000 *	0.890	0.153
	D600 - F2	-0.101	0.990	1.000 *	0.031

F2: weekly fertilization; F1: fortnightly fertilization; D450; D600: tanks fertilized respectively at 450 and 600 g/l of hen droppings. ** Significant correlation (P <0.01); * Significant correlation (P <0.05).

strongly correlated (P <0.05) with the survival rate of the post-larvae from the fertilized tanks at 600 g/m^3.

Discussion

Evolution of the Density of Zooplankton

During this test, the evolution of the zooplankton density showed rapid growth from the insemination of the zooplankton until the introduction of the fish post-larvae, which corresponds to the usual scheme described by [9] and [11]. It then dropped rapidly from day 15 before stabilizing from day 21. The drop in zooplankton density on the 15th day would no doubt be due to the predation action exerted by the newly introduced post-larvae (at 12th day) on them.

Total Length

During this test, the total length (LT) values ranged from 29.10 and 36.20 mm. These values are comparable to the respective values of 30 and 25 mm reported by [14] in Clariidae and [14] by in post-larvae of *Cirrhius mrigala* (20 days old). They nevertheless remain well below the values (44-50 mm) reported by [15] in post-larvae of *Labeobarbus batesii* aged 45 days. The differences in this result would be explained not only by the species used but also by the more intermediate duration of the nursery which was 30 days in this test.

Bodyweight, Weight Gain and Average Daily Gain

The values obtained during this work included between 420-620 mg; 180-431 mg and 12-29 mg/day respectively for bodyweight, weight gain and average daily gain were very low compared to those obtained after 45 days of nursery by [15] in the *Labeobarbus batesii* post-larvae (675-1275 mg; 525-1126 mg and 12 -26 mg/day respectively) feed on different types of food. The same observations were made by [16] in ornamental fish *Puntus vittatus* (370- 840 mg and 5 - 11.2 mg/day respectively for weight gain and average daily gain) after 75 days of nursery. These values are also much lower than those reported by [17] in *Cyprinus carpio* fingerlings (7680- 13410 mg; 170-300 mg/day respectively for weight gain and average daily gain) after 45 days of breeding and by [19] in *Osteochilus vittatus* juveniles of (13740-20690 mg and 2560-3110 mg/day) for the same parameters. The same is true for the work of [19] in the *Aspikutum* hybrid with respective values of weight gain and average daily gain of 5190-8620 mg and 280- 460 mg/day. These differences could be explained by the species used, the duration of production and the stage of breeding.

Specific Growth Rate and Condition Factor K

The specific growth rate between 3.6 and 10.85% obtained during this work is high compared to that (1.4-2.04%) reported by [20] with the post-larvae of the keurelian fish *Tor tamba*, as well as that (3.33-4.72%) obtained by [15] in post-larvae of the African carp *Labeobarbus batesii*. The same trend was observed by [21] in post-larvae of *Pethia reval* (1.3-1.49%). These differences are essentially linked to the genetic determinism of the species. The values of the condition factor K comprised during this test between 0.92 and 1.24 were comparable to the values (0.89-1.096) obtained by [22] in the wild milieu with *Labeobarbus batesii*. This value remains relatively high compared to that (0.73-0.77) obtained with the *Aspikutum* hybrid by [19]. This factor also remains very low compared to the values of 0.87-3.14 obtained by [23] with *Garra ruffa*. The low values of K observed could be explained by the high density used in this test, which was 100 individuals/m^2. In short, regardless of the level and frequency of fertilization, the fish showed good nutritional and health status since almost all K values remained greater than 1 (K≥1).

Post-Larval Survival Rate

The values of this parameter during the test varied significantly between 29.6 and 37%. This result is lower than that observed with *Cirrhius mrigala* by [1], which reports survival rates varying from 30-50% during the first 20 days of nursery and 60-70% during the 2-3 months of pre -grossing in this species. However, it remains very low compared to those (79-92% and 78-93%) obtained with the fry of *Poecilia reticulata* and *Pethia reval*, respectively by [24] and [21]. This variation could be explained by the difference in development stage considered and therefore due to the fragility and difficulty of nutrition of the post-larva used in breeding.

Conclusion

At the end of this work on the effect of the level and frequency of fertilization with chicken droppings on the post-larval growth performance of *Cyprinus carpio*, we can conclude that both the level and the frequency of fertilization had a significant effect on the zooplanktonic density and the growth performance of the common carp post-larvae. Thus, the highest values were obtained in the batches fertilized at 600 g/m^3 at a fortnightly frequency followed by those fertilized at 450 g/m^3 at the same frequency. Although the survival rate was not significantly influenced. Fertilization at 600 g/m^3 is therefore recommendable every two weeks.

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