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Effect of Two Types of Fertilizer on some Ecological Features of water, Survival and Growth of *Oreochromis niloticus* Associated with *Clarias gariepinus* in Rice-Fish Farming

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Abstract

In order to contribute to the improvement of the productivity of rice-fish farming systems, the evaluation of growth performances and the survival rate of *Oreochromis niloticus* and *Clarias gariepinus*, through the use of local organic fertilizers in comparison with mineral fertilization have been carried out. The trial was conducted in the Western Highlands of Cameroon between May and October 2022. Thus, 3 treatments namely TB (cow dung at 1000 kg/h/week), TU (urea at 80 kg/ha) and a control (T0, i.e. non-fertilized), were repeated twice in a complete random design. Physico-chemical parameter readings were taken every months and periphyton samples collected at the end of the test. A sample of 30% of the total number of each species of fish was weighed and measured at the start and end of the test. Ultimately, fertilization with cow dung leads to a drop of oxygen levels (2.94 mg/l) as well as nitrogenous nutrient salts and an increase of water conductivity. Regarding periphytons, 6 genera divided into 3 families have been identified. The density was influenced ($p<0.05$) by the 2 fertilizers and was higher ($p<0.05$) with cow dung (35.105) than with urea (1.4*106) and the controller (2.21*106). Fertilization affected ($p<0.05$) the survival of *C. gariepinus* (84% with cow dung against 64% with urea), but not that of *O. niloticus*. The highest ($p<0.05$) growth performances were obtained whatever the species in the batches having received cow dung. Gains of 0.40 and 1.03 g/D with cow dung and 0.31 and 0.64 g/D with urea respectively for *O. niloticus* and *C. gariepinus* were obtained. Concerning the yields, the speculations (rice-fish), they were significantly higher with cow dung irrespective of the speculations. Also, and regardless of speculation, batch fertilized with cow dung had the significantly highest yields (fish and rice).

Keywords: *Oreochromis niloticus*; *Clarias gariepinus*; Periphyton; Growth performances; Survival; Rice-fish systems

Introduction

Aquaculture production of food fish in sub-Saharan Africa remains very modest (i.e. 0.8% of global aquaculture production) [1]. This low production is attributable, among other things, to production costs that are too high for fish farmers, a lack of knowledge of the development of the species raised (in various systems) and a lack of popularization of farming techniques [2]. According to [3], the major constraint to the emergence of fish farming in developing countries is the cost of feed. Indeed, it represents approximately 50% of the production cost of farmed fish. So, the development of an inexpensive fish production method in controlled and semi-controlled environments with an optimization of the use of natural resources is one of the major concerns in fish farming [2]. In this situation, integrated production systems (agriculture-fish farming) can be an interesting alternative [4].

Integrated mammal-fish, poultry-fish, rice-fish farming, as well as pond cropping practices have been widely developed and practiced in countries such as China, Hungary, Germany, Malaysia, India and Cameroon [4,5]. However, associations with plants, particularly rice and fish, are more numerous and give significant yields [4-6]. According to [7], this association bodes well for good symbiotic relationships in which fish and rice derive the resources necessary for their growth. Indeed, the rice field would generate the development of an algal diversity, mainly made up of cyanobacteria [8]. These systems are generally extensive or semi-intensive where improvements are made through fertilization. Fertilizers are mineral or organic products whose use is intended to maintain or improve the primary production of the pond, as well as the physical, chemical and biological properties of the environment [9]. Chicken droppings are probably the most widely used organic fertilizer in ponds. However, its availability is becoming increasingly rare due to the strong demand for other agricultural speculations. As a result, producers generally resort to other types of manure and especially today those which are the least competitive and available at a lower cost. However, the evaluation of their real effects on the productivity of these systems remains poorly known in our local

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contexts. It is in this perspective that the overall objective of this study was to contribute to the improvement of the productivity of rice-fish farming systems through the evaluation of the influence of urea and cow dung on its production. More specifically, it was a question of assess the influence of cow dung and urea on the ecologicals features of water (physicochemical characteristics periphyton's production of water), on the survival and growth performances of *Oreochromis niloticus* and *Clarias gariepinus* and on the yield of rice-fish-systems.

Material and Methods

Study area

The study was conducted from May to October 2014, in the village of Ngoundoup located between 5°4' North latitude and 10°49' East longitude. It is located in the Western Highlands of Cameroon, more precisely in the District of Koutaba, Noun's Division, at an altitude of 1168 m. Temperatures range between 19°C and 29°C with an average of 22°C and relative humidity generally above 60%. The annual rainfall varies between 1500 and 2000 mm, with a rainy season that extends from mid-March to mid-November. In this area, rice cultivation can be done twice a year.

Biological material

The test was carried out with 900 male fingerlings of *Oreochromis niloticus* (15 ± 2 g) and 300 *Clarias gariepinus* (9 ± 2 g), all reproduced at the Research Station from IRAD in Koupa-Matapit (Foumban). The plant material consisted of 14400 rice seedlings of the NERICA 56 variety from a nursery set up on the study site.

Technical material

The technical equipment was composed of breeding structures (7 fish ponds of 200 m2 each built in derivation), an ichthyometer (1 mm) to measure the sizes of the fish and the height of the water, an electronic scale portable Sartorius brand (0,01 g) used to weigh fish and rice in paddy. The physical and chemical parameters of the water (pH, dissolved oxygen, water temperature, conductivity) were measured using the devices indicated in Table 1. 250 ml double cap polyethylene bottles were used to collect pond water for the determination of nitrite (NO2), nitrate (NO3-), ammonium (NH4+) and orthophosphate (PO43-) ions and their analysis was carried out using a spectrophotometer.

Setting up the test

The setting up of the trial began on May 9, 2022 with the rice nursery, which lasted 32 days in a pond near the site; followed by the mud filling and transplanting of the ponds chosen according to the following technical itinerary: The treatments were randomly tested from June to October 2022 in two replicates in 6 ponds (Figure 1). The loading of *O. niloticus* fingerlings took place 31 days after transplanting the rice as soon as the water level gradually rose and 61 days for *C. gariepinus*. Stocking density was 1 fish/m² (0.75 Tilapia and 0.25 Clarias/m²). Total fish biomasses per pond were recorded at the start of the test. The ponds were seeded with NERICA 56 at a density of one foot every 20 cm, thus, 2400 seedlings per pond [2]. All ponds were transplanted at the same time.

Conduct of the test

The conduct of the trial consisted of additional fertilization depending on the treatments which began 7 days after stocking, and was done once again at the end of the second month with urea (100 kg/ha) and monthly with cow dung at a dose of 1000 kg/ha/week [10].

Collection of Data

Table 1: Means and standard deviations of the common physicochemical characteristics of water according to the types of fertilizer.

Physico-chemical characteristics	Types of fertilizer		
	Witness	**Dung**	**Urea**
Temperature (°C)	22.62 ± 0.37^{a}	22.47 ± 0.58^{a}	22.42 ± 00.49^{a}
pH	07.22 ± 0.33^{a}	07.23 ± 0.53^{a}	07.15 ± 00.43^{a}
Oxygen (mg/l)	05.26 ± 2.a23^{a}	02.94 ± 1.04^{b}	04.70 ± 01.66^{a}
Conductivity (µs/cm)	55.45 ± 16.17^{c}	114.59 ± 49.99^{a}	83.73 ± 39.12^{b}
Depth (cm)	31.28 ± 8.11^{a}	34.85 ± 15.46^{a}	31.73 ± 11.75^{a}
a,b,c: values with the same letter on the same row do not differ significantly (P>0,05).			

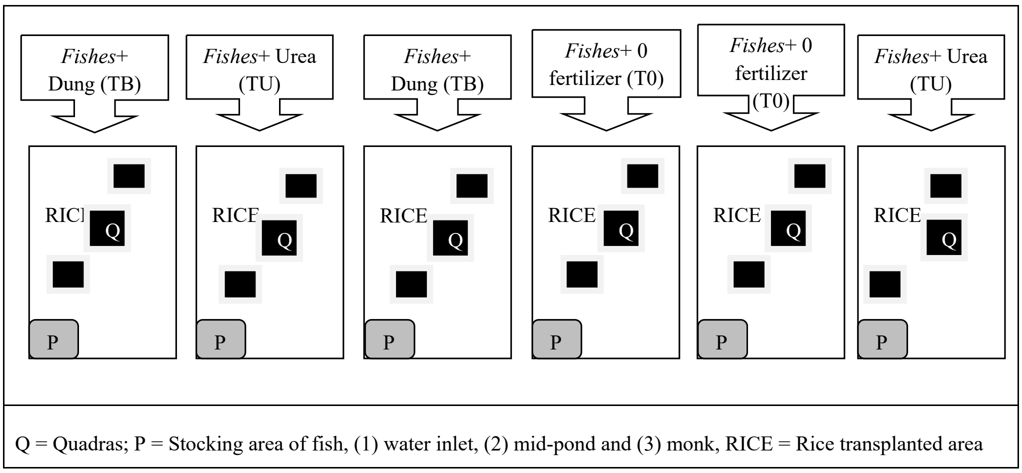


Figure1: Diagrams of the experimental device.

Physical and chemical parameters of water

The measurements of the physical and chemical parameters of the water (transparency, pH, temperature, dissolved oxygen and conductivity) were made in situ, on a monthly basis between 6 a.m. and 8 a.m. in each pond. In addition, water samples were taken from flasksin 250 ml double-capped polyethylene bottles then transported in a refrigerated cooler to bestore at a temperature below 4°C.The concentration of dissolved inorganic nitrogen [nitrite (NO^{2-}), nitrate (NO^{3-}), ammonium (NH_4^+)] and dissolved orthophosphate (PO_4^{3}) was determined according to the standard AFNOR 93 method (spectrophotometry). For these salts, only two samples were analyzed (45th and 90th day).

Collection and identification of periphyton

Periphyton samples were collected at the end of rearing on rice tillers of the same diameter, at the entrance, in the middle and at the exit of the ponds. This was done by scraping the entire portion of the tiller using a knife. The samples of 25 ml each were labeled and stored in plastic boxes (with film) at a proportion of 3/4 of the sample ¼ of 5% formalin. Settings between slide and coverslip were made for observation (at 40X magnification). Periphyton taxa were identified using specialized works [11-13] using a MOTIC binocular microscope. The richness in organism category as well as the densities by species and total were calculated.

Biotic parameters (rice and fish)

A sample of 30% of the total number of each species of fish was weighed and measured at the start and end of the test. Total and standard weights and lengths were determined to the nearest gram and centimeter, respectively. Dead fish were located and counted during and at the end of the test. At the rice level, three quadrats of 1 m² (at the water inlet, in the middle and at the monk) were defined in each pond (Figure 1). The data concerning the rice yield in paddy were raised at the harvest of the rice in the three quadrats and the total quantity calculated.

Data analysis or calculated

Body mass gain: It is calculated for each species using the following formula: GPM (g) = Final average weight (g) - Initial average weight (g).

Average daily gain (ADG): this parameter makes it possible to evaluate the daily weight gain and is calculated according to the formula: GMQ = **ADG** (g)/D where GPM is the average weight gain in g and D is the rearing duration in days for each species.

Specific growth rate (SGR): It is calculated for each species from the following formula:

$$\mathbf{SGR}\ (\%) = 100 \times \frac{\ln Pm_f - \ln Pm_i}{d}$$

Condition factor (K): This coefficient was calculated for each fish individually according to the formula: K=100 x Pf/Lt³; with: Pf: final weight of the fish (g) and Lt: total length of the fish (cm).

Survival rate (*S*): It is calculated according to the relationship: ***S* *(%) = (Nf/Ni) *100,*** with: Ni = initial number of fish; Nf = number of fish at the end of rearing. Tilapia fingerlings were not taken into account.

Fish yield or production per hectare per year: Production/ha/year = ((Production (kg)/area) *10000) *2.

Rice data: Rice paddy yield or paddy production per hectare = (Dried production (kg)/area) *10000.

Statistical analyzes of data

The physicochemical characteristics and the agronomic performances of the various systems were subjected to the one-factor variance analysis to test the effects of the treatments. When they were significant differences, the Duncan test at 5% allowed us to compare the means. SPSS 20.0 software was used for these analyses.

Results

Effect of fertilizer's type on ecological features of water

1.1. Physicochemical characteristics and nutrient salts

- **Physicochemical characteristics**

The averages of the physicochemical parameters of the water according to the type of fertilizer are presented in Table 1. It emerges from this that, with the exception of dissolved oxygen and conductivity, which varied significantly ($p<0.05$), all other characteristics were comparable ($p>0.05$).

- **Nutrient salts of water**

The average nutrient salt contents of the water according to the types of fertilizers summarized in Table 2 indicate that, the concentrations of ammonium, nitrate and nitrite (0.35 mg/l) were significantly higher ($p<0.05$) in basins treated with urea, unlike phosphate, which content was lower.

1.2. Effects of fertilizers on the diversity and density of periphytons

- **Diversity of periphytons**

Table 3 presents the diversity and relative abundance of the different taxa observed. This table shows 6 genera of periphytons divided into three families. So the type of fertilizer does not affect the diversity of periphytons.

- **Periphyton density**

Figure 2 illustrates the effect of fertilizers on the average densities and indices of the presence of periphytons.

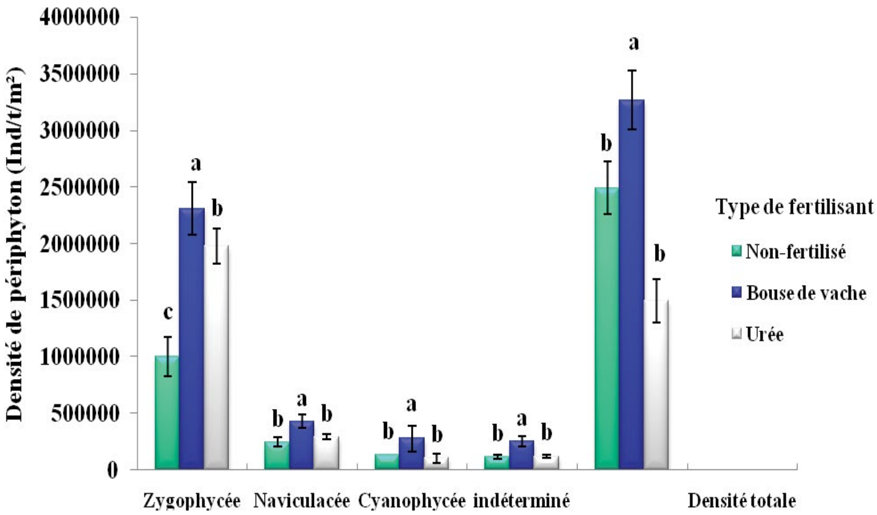
It follows from figure 2 that there was a significant development

Table 2: Means and standard deviations of nutrient salts in water according to the types of fertilizer.

Chemical characteristics	Types of fertilizer		
	Witness	Dung	Urea
NH4+(mg/l)	0.23 ± 0.20^{b}	0.40 ± 0.38^{b}	0.82 ± 0.65^{a}
NO3-(mg/l)	0.11 ± 0.10^{b}	0.19 ± 0.15^{b}	0.40 ± 0.34^{a}
NO2-(mg/l)	0.02 ± 0.01^{c}	0.13 ± 0.12^{b}	0.35 ± 0.30^{a}
PO43-(mg/l)	0.06 ± 0.00^{b}	0.21 ± 0.12^{a}	0.04 ± 0.01^{b}
a, b, c: bars of the same color bearing the same letter do not differ significantly (P>0,05).			

Table 3: Effect of fertilizer types on the diversity and presence index of periphytons.

Diversity of periphytons		**Type of fertilizer**		
Families	**Genders**	**Witness**	**Dung**	**Urea**
Zygophycea	Closterium	+	+++	+
	Actinoteium	+++	+++	++
	Cosmorium	+++	+++	++
Naviculaceae	Navicula	+	++	++
	Pinnularia	+	+	+
Cyanophyceae	Microcystis	+	+	+
Indeterminate	Non-filamentous	+	++	+
	filamentous	+	+++	++
+: weak, ++: strong, +++: very strong				



a, b, c: bars of the same color bearing the same letter do not differ significantly (P>0,05).

Figure 2: Density and presence index of periphytons depending on the type of fertilizer.

of periphytons independently of the fertilizer. This development has resulted in a significantly ($p<0.05$) higher density with cow dung (3267156.66 ind/m²); showing that this medium is favorable for the growth of *Oreochromis niloticus* which ingests these periphytons by browsing. Furthermore, the Zygophyceae family appears to be more abundant regardless of the fertilizer, which would be explained by the low temperature of the environment.

1. Effect of fertilizer type on growth and survival of *Oreochromis niloticus* **and** *Clarias gariepinus*

1.1. Growth's performances and survival rate of *Oreochromis niloticus*

- **Growth's performances of *Oreochromis niloticus***

Table 4 below summarizes the growth parameters of *Oreochromis niloticus* according to the type of fertilizer. Thus, weight parameters (live weight, average daily gain, specific growth rate) were significantly higher ($p<0.05$) with cow dung, while total length and condition factor K were comparable.

- **Survival rate of *Oreochromis niloticus***

Figure 3 illustrating the survival rate of *Oreochromis niloticus* does not indicate a significant difference ($P>0.05$) whatever the fertilizer. However, the highest value was obtained with cow dung.

1.2. Effect of fertilizer type on growth performances and survival rate of *Clarias gariepinus*

- **Growth performances of** *Clarias gariepinus*

The means of the growth parameters of *C. gariepinus* according to the type of fertilizer are summarized in Table 5. It appears that the values of the various growth parameters in an environment fertilized with cow dung are significantly ($p<0.05$) higher compared to those of urea-fertilized and non-fertilized media.

- **Survival rate of *Clarias gariepinus***

The survival of *Clarias gariepinus* is presented in figure 4. It appears that this survival was comparable in the fertilized media and significantly lower ($p<0.05$) in the unfertilized batches. Furthermore, the highest value was obtained with cow dung.

2. Agronomic yields of systems

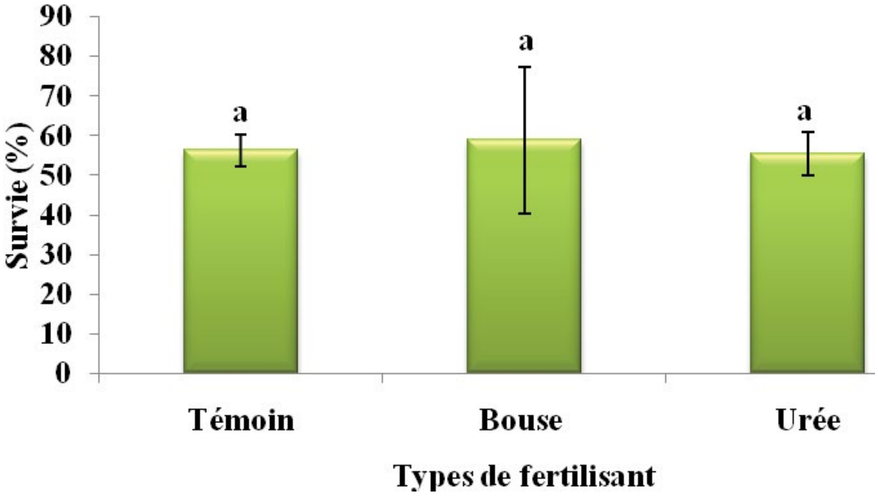
Table 6 below summarizes the agronomic yields of the different systems. It emerges that, whatever the speculation (rice-fish), the significant ($p<0.05$) highest yields were obtained with cow dung while

Table 4: Means and standard deviations of growth performance of Oreochromis niloticus according to the types of fertilizer.

	Type of fertilizer		
Settings	**Witness**	**Dung**	**Urea**
Total length (cm)	12.88 ± 1.44^{a}	14.35 ± 2.71^{a}	12.95 ± 2.13^{a}
Final weight (g)	41.15 ± 1.89^{b}	56.95 ± 21.57^{a}	44.95 ± 7.08^{b}
Weight gain (g)	25.35 ± 2.99^{b}	41.15 ± 21.86^{a}	29.15 ± 7.11^{b}
ADG (g)	00.30 ± 0.13^{b}	00.40 ± 0.22^{a}	00.31 ± 0.07^{b}
Specific growth rate (%)	01.01 ± 0.15^{b}	01.29 ± 0.39^{a}	01.10 ± 0.20^{b}
Condition factor K (g/cm^{3})	02.07 ± 0.82^{a}	02.28 ± 0.78^{a}	02.28 ± 0.92^{a}
a,b: values with the same letter on the same row do not differ significantly (P>0,05).			

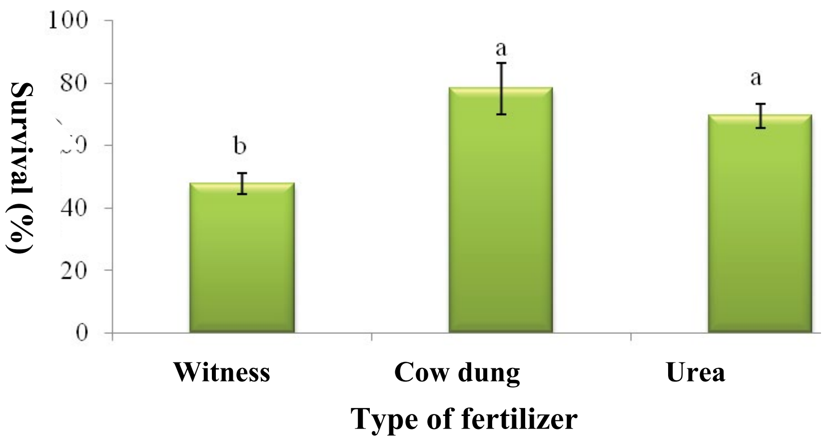
Table 5: Means and standard deviations of growth parameters of Clarias gariepinus according to the types of fertilizer.

Settings	Types of fertilizer		
	Witness	**Dung**	**Urea**
Total length (cm)	14.63 ± 1.57^{b}	18.05 ± 1.95^{a}	16.79 ± 1.16^{a}
Standard Length (cm)	13.60 ± 2.11^{b}	16.08 ± 2.11^{a}	15.04 ± 1.90^{b}
Final weight (g)	44.10 ± 6.52^{b}	70.40 ± 8.20^{a}	46.80 ± 2.93^{b}
Weight gain (g)	35.60 ± 6.55^{b}	62.00 ± 7.98^{a}	38.30 ± 2.87^{b}
ADG (g)	00.59 ± 0.11^{b}	01.03 ± 0.17^{a}	00.64 ± 0.05^{b}
Specific growth rate (%)	02.74 ± 0.29^{b}	03.43 ± 0.39^{a}	02.85 ± 0.18^{b}
Condition factor K (g/cm^{3})	01.45 ± 0.35^{a}	01.15 ± 0.25^{b}	00.94 ± 0.17^{b}
a,b: values with the same letter on the same row do not differ significantly (P>0,05)			



a: bars with the same letter do not differ significantly (P>0,05)

Figure 3: Survival rate of Oreochromis niloticus according to fertilizers.



a,b: bars with the same letter do not differ significantly (P>0.05).

Figure 4: Survival rate of *Clarias gariepinus* according to the type of fertilizer.

Table 6: Agronomic yields according to the type of fertilizer.

Net yield	Type of fertilizer		
	Witness	Dung	Urea
Fish yield (t/ha/year)	0.28 ± 0.01[c]	0.65 ± 0.04[a]	0.045 ± 0.02[b]
Net rice yield (t/ha)	3.40 ± 0.31[b]	4.57 ± 0.94[a]	2.40 ± 0.22[c]
a,b,c: values with the same letter on the same line do not differ significantly (P>0,05).			

the lowest yields($p<0.05$) were obtained with the batches fertilized with urea for the rice and not fertilized for the fish.

Discussion

The values of the physicochemical parameters are within the ranges tolerated by the species used in the test. However, this analysis shows us that the variations in water temperature observed in the different environments during the test are not compatible with the thermal interval favorable to better growth of *Oreochromis niloticus,* being between 24 to 28°C as indicated by [14,15] or that of *Clarias gariepinus* which is between 27.5 and 32.5°C [16]. This low value would be related to the atmospheric temperature of the study area, the average of which is 22°C. However, under the effect of sunshine, temperatures above 28°C or even 30°C were regularly obtained at the end of the day. Besides, the minimum content of dissolved oxygen observed in the morning with cow dung is below 3 mg/l, the value from which respiratory stress appears in *Oreochromis niloticus* [17]. This low oxygen level could be explained by the rapid decomposition of cow dung and the increase in the bacterial population, as demonstrated by the work of [18].The contents of dissolved nutrient compounds (NO^{3-}, NO^{2-}, PO_4^{3-} and NH_4^+) remain below the thresholds described [19] which are 2.1 mg/l for nitrates, 2.1 mg/l for nitrites, 2.3 mg/l for ammonia, 0.5 mg/l for phosphate (value not to be exceeded to avoid proliferation of algae) and 0.2 mg/l for NH_3 considered as the minimum toxicity threshold for fish [20]. These low values could be explained by the ability of plants (rice) to fix mineral elements (purification) as demonstrated by the work [21]. However, the richness of the basins treated with urea and cow dung respectively in nitrogenous and phosphate elements would be linked to the composition of the latter. In addition, the low levels of nitrogenous elements in ponds fertilized with dung corroborate the results obtained by [22,23]. To make it possible to raise the level of nitrogen, the work of [24] reveals that a weekly supply of mineral fertilizer (urea) would be profitable.

Fish survival and growth performances

The survival rates obtained were relatively high but low compared to those obtained by [5] in semi-intensive fish farming which was 60.5-100% and 66.7-100% respectively with Tilapia and Clarias. The low survival rate of the tilapias observed could be explained by the shallow depth of the ponds, which would expose the fish to predators. Also, this rate may be related, especially in batches fertilized with cow dung, to the conditions of anoxia observed.

With regard to growth parameters, the average weight (g) of tilapias and Clarias at 13.5 weeks is substantially equal to the 44 g and 66 g obtained after 17 weeks of rearing by [25] in a semi-intensive polyculture of *Oreochromis niloticus* associated with *Clarias gariepinus* in a stagnant water pond. On the other hand, the average daily gain obtained remains much lower than the 1.6 g/d obtained by [26] in rice-fish farming with *Oreochromis niloticus*. This low rate could be explained by the low values on the one hand of temperature and on the other hand of dissolved oxygen, which are also negatively correlated with this parameter. However, the particularly high growth performance in dung-fertilized ponds would be due to a greater supply provided by epibiont organisms. Moreover, this could be explained by other food sources present in the rice field, such as plankton and zoobenthos. The values of the condition coefficient K, which constitutes a good index of the physiological and nutritional state of the fish, were higher than 1 indicating overweight in fish [27]. Thus, the type of fertilizer did not act negatively on the health status of the fish.

Agronomic performance of systems

The fish yields obtained are much lower than the values of 1 to 2 t/ha/year noted by several authors in rice-fish farming with food [6,8,28]. This could be explained not only by the low temperature of the study area but also by the loading density which was low.

The rice yields obtained are in the yield range of 1 to 10 t/ha in culture with submersion [29]. However, the yield of 4.5 t/ha is higher than the values of 3.68 t/ha and 3.08 obtained by [30] respectively in rice-fish and rice-growing environments. On the other hand, these yields are far lower than the 7.5 t/ha obtained in intensive rice cultivation with fertilization by [30]. This low yield would be, in part, linked to the average water heights of the ponds which are almost all higher than the threshold of 1/5 (0.20) of the height of the rice stem, which would have hindered the photosynthetic activity of the rice during its cycle. This same observation was made by [31,32] in 1989 in a study on the conditions for improving rice-fish farming and corroborates the results obtained [2]. However, these yields are higher for all the combinations than the yield of 1.7 t/ha regularly obtained by producers in the same zone. This improvement could be linked to the ecological network created by the association of rice and fish. However, urea performed poorly compared to ponds fertilized with dung and even to controls. This corroborates with the results obtained by [32] who showed that high doses of mineral fertilizers (≥75 kg/ha) would have a depressing effect on yields and even compares this to a case of rice pathology.

Conclusion

At the end of this work on the study of the effect of two types of fertilizer on the ecological water, survival and growth of *Oreochromis niloticus* associated with *Clarias gariepinus* in rice-fish farming, the main conclusions were as follows: With the exception of water temperature and pH, the effect of fertilizers was significant on conductivity, dissolved oxygen and all nutrient salts. Thus, the values of nitrogenous nutrient salts were higher with urea, unlike phosphate, which was higher with cow dung. Regarding survival, the type of fertilizer did not act significantly on tilapia. Unlike *Clarias gariepinus,* whose rate was significantly higher with cow dung? Fertilizer type significantly influenced growth characteristics except for condition factor K and lengths, and the highest values were obtained regardless of species with cow dung. Also, and regardless of speculation, batch fertilized with cow dung had the significantly highest yields (fish and rice).

Data Availability Statement

The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical Requirements and Consent to Participate

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals.

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