Article

Multispecies Fresh Water Algae Production for Fish Farming Using Rabbit Manure

Adandé Richard 1,*, Liady Mouhamadou Nourou Dine 1, Djidohokpin Gildas 1, Adjahouinou Dogbé Clément 1, Azon Mahuan Tobias Césaire 1, Micha Jean-Claude 2 and Fiogbe Didier Emile 1

1 Laboratory of Research on Wetlands (LRZH), Department of Zoology, Faculty of Sciences and Technology, University of Abomey-Calavi, B.P. 526 Cotonou, Benin; liadynouroudine@gmail.com (L.M.N.D.); gdjidohokpin@gmail.com (D.G.); adjaclem@gmail.com (A.D.C.); azonmahuan@yahoo.fr (A.M.T.C.); edfiogbe@yahoo.fr (F.D.E.)

2 Department of Biology, Research Unit in Environmental Biology, University of Namur, 5000 Namur, Belgium; jean-claude.micha@unamur.be

* Correspondence: richard_adande@yahoo.fr; Tel.: +229-95583595

Received: 27 July 2020; Accepted: 19 October 2020; Published: 30 November 2020

Abstract: The current study aims at determining the optimal usage conditions of rabbit manure in a multispecies fresh water algae production for fish farming. This purpose, the experimental design is made of six treatments in triplicate including one control T0, T1, T2, T3, T4, T5 corresponding respectively to 0, 300, 600, 900, 1200, 1500 g/m3 of dry rabbit manure put into buckets containing 40 L of demineralized water and then fertilized. The initial average seeding density is made of 4 × 103 ± 2.5 × 102 cells/L of Chlorophyceae, 1.5 × 103 ± 1 × 102 cells/L of Coscinodiscophyceae, 3 × 103 ± 1.2 × 102 cells/L of Conjugatophyceae, 2.8 × 103 ± 1.5 × 102 cells/L of Bascillariophyceae, and 2.5 × 103 ± 1.4 × 102 cells/L of Euglenophyceae. During the experiments, the effects of these treatments on abiotic and biotic parameters (chlorophyll-a concentration, phytoplankton density and algal density) of different production media were monitored. Results show that average density of different phytoplankton classes is higher in treatment T5 (7.91 × 108 ± 6.78 × 107 cells/L) followed by T4 (5.56 × 108 ± 4.27 × 107 cells/L), T2 (3.87 × 108 ± 3.10 × 108 cells/L), T3 (3.79 × 108 ± 3.18 × 108 cells/L, with high significant difference (F (4,84) = 5.35, p < 0.00). Chl-a concentration varied from 0.07 ± 0.05 mg/L (T0) to 14.47 ± 12.50 mg/L (T5) with high significant differences observed among treatments (F (5,83) = 3,09, p = 0.01). In addition, fourteen (14) species belonging to eight (8) families, five (5) classes and three (3) phyla were identified in our different production media. During the culture, Chlorophyceae class was the most represented in all treatments with 5 species (36% of the specific diversity) while Euglenophyceae class (7%) was the least represented with only one (01) species. According to these results, treatments T2 (600 g/m3), T3 (900 g/m3) and T4 (1200 g/m3) of dry rabbit manure are those worthy to be recommended as an alternative for a low cost massive production of multispecies freshwater algae that can be easily used by freshwater zooplankton and macroinvertebrates. Indeed, despite the best performances that it shows, treatment T5, presents important eutrophication’s risks.

Keywords: aquaculture; multispecies algae; rabbit manure; production; zooplankton

1. Introduction

Animal wastes are valorized in aquaculture as fertilizers to increase halieutic production in order to meet the crescent fish demand observed these latter years [1–4]. Due to their low cost and low pollution risks when recycling and treatments cautions are taken off stream of production
system, certain animal wastes such as rabbit manure whose mineral salts richness is well known are used at the detriment of chemical fertilizers to increase primary production in fish farming systems in order to produce fresh water zooplankton and benthic macro-invertebrate that constitute the main food source for fish larvae and fries [5–9]. In some western countries, modern technologies are used to produce important algae biomass for fish farming from these wastes [10], unfortunately these technologies are often highly expensive and not adapted to developing countries. In developing countries where sun light and temperature are naturally high to favor the utilization of mineral salts (ammonium, nitrate, nitrite and orthophosphate) by algae, simple and low cost algal production techniques from animal wastes can be used in fish farming [4,8,9,11]. Algae are food for zooplankton and benthic macro-invertebrates that must be rich in nutrients (lipids, poly-unsaturated fatty acids, polysaccharides, carotenoids, steroids, and premium vitamins for fish growth [12] in order to guarantee a good fish production. However, very few studies in developing countries are focused on the improvement of plankton culture [1,13] though judicious use of various sources of animal genuine organic fertilizers could enable important production of algae at low cost to feed zooplankton and benthic macro-invertebrates in order to optimize profitability in rural fish farming [9,14]. The nutritional quality of plankton is influenced by fertilization and the water quality and determines the composition of phytoplankton species in culture media [1,15], and is the reason why the aim of this study is to determine the optimal dose of rabbit manure for multi-species production of fresh water algae for fish farming.

2. Material and Methods

2.1. Experimental Design

The experimental design comprises 18 plastic buckets of 80 L capacity (previously cleaned and disinfected), exposed to the opened air at the Research Station for Fish Farming Diversification of the University of Abomey-Calavi (UAC). These buckets are filled with 40 L of demineralized water immediately fertilized with manure of rabbit fed on cheap price food diet made of 2% cassava, 30% corn bran, 10% palmist cake, 10% soya cake, 5% cotton cake, 2% shell, 10% malt, 5% beer yeast, 10% Panicum maximum, and 1% of salt [14]. Six (6) treatments were tested in triplicate: T0 (the control), T1, T2, T3, T4, and T5 corresponding respectively to 0, 300, 600, 900, 1200, 1500 g of dry rabbit manure (RM) in one (1) m³ of water. These manures are made of 15.02% of Nitrogen (N), 1.26% of phosphorus (P) and 0.84% of potassium (K) with ratio N/P = 11.89.

2.2. Seeding, Identification and Counting of Phytoplankton

At the beginning of the experiment, production media (water) containing rabbit manure was previously abandoned for three days to enable fertilization by nutrients contained in rabbit manure, and then they were seeded with phytoplankton. Seeding has been achieved by sampling 10 L of water from multi-culture (Clarias gariepinus and Oreochromis niloticus) pond and gently filtering it according to Agadjihouédé et al. [16] by using a 25-µm plankton net in order to eliminate zooplankton, then the obtained filtrate was added to culture medium in each bucket for phytoplankton seeding. A fresh part has been used for observation and identification of phytoplankton species with photonic binocular microscope (BI 100; VWR International Belgium) and another 100 mL part was treated with formaldehyde 5% and concentrated 100 times for phytoplankton counting using a Neubauer counting cell under a photonic microscope [17]. Identification was carried out according to Adjahoinou et al. [17] from photographs realized at 10×, 40× and, 100× according to height and mobility of algae species and by using the identification keys of Bourrelly [18–20], Compère [21–26], Iltis [27], and Guiry et al. [28]. Algal density was estimated according to Bouali et al. [29] through the equation:

\[ D \text{ (cells/L)} = M \times \frac{C}{V} \times 10^5 \]

with M: mean algae cell number per rectangle of hematimeter, C: concentration coefficient of the ampoule, V: sample volume. Thus, the initial mean seeding density was \( 4 \times 10^3 \pm 2.5 \times 10^2 \) cells/L of Chlorophyceae, \( 1.5 \times 10^3 \pm 1 \times 10^2 \) cells/L of Coscinodiscophyceae, \( 3 \times \)
10^3 ± 1.2 × 10^2 cells/L of Conjugatophyceae, 2.8 × 10^3 ± 1.5 × 10^2 cells/L of Bacillariophyceae and 2.5 × 10^3 ± 1.4 × 10^2 cells/L of Euglenophyceae.

During experiment monitoring, for each phytoplankton sampling, the culture medium was homogenized, then 5 L of water were filtered with a 25-µm plankton net and concentrated 100 times.

2.3. Monitoring of Physico-Chemical and Trophic Parameters

During the experiment, temperature (°C), pH, conductivity (µs/cm), and dissolved oxygen (mg/L) were measured in situ every three days at 12 PM with a CALYPSO, Champigny-Marne, France multi-parameter device, sensitivity ±0.1 °C (Version Soft/2015, SN-ODEOA 2138). At each sampling, 1/2 L of the culture medium was filtered for determination of chlorophyll-a (Chl-a) content, algal biomass, and nutritive dissolved salts concentrations (ammoniac nitrogen, nitrate, nitrite, and orthophosphates). The dosage of Chl-a was carried out according to the protocol described in AFNOR NF T90-117 standards. Nutritive salts were determined according to Rodier et al. [30] using a HACH DR/2800 molecular absorption spectrophotometer.

2.4. Statistical Analyses

Statistical analyses were carried out with STATISTICA software (Statsoft inc., Tulsa, OK, USA) at a threshold of 5%. A general linear model (GLM) was used followed by the Bonferroni test to compare the means of phytoplankton densities. The effect on different physicochemical parameters were analyzed using repeated measures ANOVA after checking the sphericity conditions. The values reported in the results are means and standard deviations. The correlation between the concentration of chl-a and the treatments was analyzed using the Pearson correlation test.

3. Results

3.1. Abiotic Parameters

Except temperature, mean values of physico-chemical parameters varied from one treatment to another from 22.12 to 50.63 for conductivity, from 0.72 to 1.05 for pH, from 0.72 to 2.95 for dissolved oxygen, from 0.01 to 0.03 for salinity, from 8.47 to 27.11 for TDS, and from 0.37 to 1.51 for transparence. As illustrated in Table 1, mean values of pH, electrical conductivity, salinity, and TDS were higher in fertilized media than in control treatment T0 with high significant difference (p < 0.00). Except pH, the highest mean values of different parameters prior quoted were recorded in medium T5. The same remark was made for dissolved oxygen though concentration was most important in treatments T2 and T5, followed by T4 and T3 with significant difference (p < 0.05). Transparence decreased progressively from control medium T0 to the most fertilized medium T5.

| Parameters | T0 | T1 | T2 | T3 | T4 | T5 | F       |
|------------|----|----|----|----|----|----|---------|
| T°C        | 33.74 ± 1.70 | 34.02 ± 1.81 | 34.30 ± 1.70 | 34.34 ± 1.87 | 34.48 ± 1.86 | 34.51 ± 1.78 | F(5, 12) |
| Cond (µs/cm) | 1.70 | 1.81 | 1.70 | 1.87 | 1.86 | 1.78 | F(5,12) = 12.63 |
| pH         | 37.66 ± 23.17 | 597.82 ± 34.10 | 3604.50 ± 23.17 | 3607.19 ± 22.12 | 3625.58 ± 22.12 | 3664.45 ± 22.12 | F(5,12) = 41.86 |
| DO (mg/L)  | 6.87 ± 0.94 | 7.29 ± 0.79 | 7.33 ± 0.94 | 7.12 ± 0.92 | 7.09 ± 1.05 | 7.16 ± 1.05 | F(5,12) = 5.53 |
| Sal (mg/L) | 0.08 ± 0.01 | 0.31 ± 0.02 | 0.32 ± 0.01 | 0.32 ± 0.01 | 0.33 ± 0.01 | 0.36 ± 0.03 | F(5,12) = 5.33 |
| TDS        | 64.60 ± 8.77 | 296.92 ± 17.07 | 302.56 ± 8.77 | 303.43 ± 7.47 | 318.76 ± 16.81 | 338.61 ± 27.11 | F(5,12) = 9.33 |
| Transp (cm) | 8.77 | 17.07 | 8.77 | 7.47 | 16.81 | 27.11 | 7.88 |

Table 1. Physico-chemical parameters of the different treatments.
Cond: conductivity, DO: dissolved oxygen, Sal: salinity, Transp: transparency, TDS: total dissolved solids. T0 (the control), T1, T2, T3, T4, T5 corresponding respectively to 0, 300, 600, 900, 1200, 1500 g of dry rabbit manure (RM). Mean values affected by different letters on the same line are significantly different at 5% threshold. Mean concentrations of nutrients varied significantly ($p < 0.05$) from one treatment to another (Figure 1). The lowest mean values ($0.06 \pm 0.12$ mg/L; $0.07 \pm 0.11$ mg/L; $0.08 \pm 0.10$ mg/L and $0.5 \pm 0.13$ mg/L respectively for N-NO$_3$, N-NO$_2$, N-NH$_3$, and P-PO$_4^{3-}$ were obtained in the control treatment (T0) while the highest ($2.82 \pm 0.75$ mg/L; $0.75 \pm 0.35$ mg/L; $0.81 \pm 0.14$ mg/L and $3.15 \pm 0.16$ mg/L) were recorded in treatment T5.

**Figure 1.** Mean concentrations of nutrients in the different culture media. For the same parameters, means followed by the same letters are not significantly different ($p > 0.05$). T0 (the control), T1, T2, T3, T4, and T5 correspond, respectively, to 0, 300, 600, 900, 1200, 1500 g of dry rabbit manure (RM).

3.2. Biotic Parameters

3.2.1. Chlorophyll-a Concentrations

Figure 2 shows that mean concentrations of chl-a obtained during the experiment varied from $0.07 \pm 0.05$ mg/L (T0) to $14.47 \pm 12.50$ mg/L (T5) with high significant difference observed among treatments ($F_{5,12}=9554.9$, $p < 0.00$). In the same way, high positive correlation ($R = 0.88$, $p < 0.00$) was observed between chlorophyll-a concentration and rabbit manure dose.

**Figure 2.** Mean concentrations of chlorophyll-a in different treatments: T0 (the control), T1, T2, T3, T4, and T5 correspond, respectively, to 0, 300, 600, 900, 1200, 1500 g of dry rabbit manure (RM).
3.2.2. Phytoplankton Diversity

A total of fourteen (14) phytoplankton species belonging to eight (8) families, five (5) classes, and three (3) phyla were identified in the production media (Table 2).

### Table 2. Phytoplankton taxons identified and produced from rabbit manure.

| Phylum          | Classes               | Families    | Genuses and Species              |
|-----------------|-----------------------|-------------|----------------------------------|
| Bacillariophytes| Coscinodiscophyceae   | Melosiraceae| *Melosira italica*               |
|                 |                       |             | *Melosira sp*                     |
|                 | Bacillariophyceae     | Pinnulariaceae| *Pinnularia viridis*             |
|                 |                       |             | *Pinnularia sp*                   |
|                 | Naviculaceae          |             | *Navicula sp*                     |
|                 | Closteriaceae         |             | *Closterium sp*                   |
| Conjugatophyceae| Desmidiaceae          |             | *Staurastrum margaritaceum*       |
|                 |                       |             | *Staurastrum pinnatum*            |
| Charophytes     |                       |             | *Cloelastrum sp*                  |
| Chlorophyceae   | Scenedesmaceae        |             | *Scenedesmus javanensis*          |
|                 |                       |             | *Scenedesmus apiculatus*          |
|                 |                       |             | *Scenedesmus quadricauda*         |
| Euglenophytes   | Euglenophyceae        | Euglenaceae | *Euglena ehrenbergii*             |
| Palmellopsidaceae|                      |             |                                  |

3.2.3. Phytoplankton Density

The evolution of phytoplankton density varies according to phytoplankton classes and applied treatments (Figures 3 and 4). During the production period, two to three peaks were observed according to the treatments. An increase in the algal density of different classes was observed from 3rd in all treatments with a first peak at the 3rd day in treatment T1 and the 6th day for treatments T0, T2, T3, T4, and T5 (Figure 3). During the peak, the highest densities were those of Chlorophyceae and Bacillariophyceae, respectively, in treatments T4 (5.14 × 10⁸ ± 1.69 × 10⁸ cells/L) and T5 (8.60 × 10⁸ ± 1.19 × 10⁸ cell/L). From the 9th day, algae density decreased in all media but growth started enabling a second peak at the 12th day, although with lower densities than the first. From the 15th day till the end of the experiment, phytoplankton density decreased in treatments T3, T4, and T5. However, a third peak was observed at the 21st day in treatments T1 and T2 with lower densities than the second and at the 24th day in treatment T0 with almost the same density as the second. On the contrary, the mean density obtained in the first and second peak in which Chlorophyceae and Bacillariophyceae were predominant, that of the third peak is dominated by Conjugatophyceae, Chlorophyceae, and Bacillariophyceae (Figures 3 and 4), respectively, in treatments T0 (3.33 × 10⁸ ± 2.21 × 10⁸ cells/L), T1 (2.40 × 10⁷ ± 1.10 × 10⁸ cells/L), and T2 (2.47 × 10⁷ ± 1.67 × 10⁸ cells/L). The mean total density for the whole phytoplankton classes during experiment period was higher in treatment T5 (7.91 × 10⁸ ± 6.78 × 10⁷ cells/L) followed by T4 (5.56 × 10⁸ ± 4.27 × 10⁷ cells/L), T2 (3.87 × 10⁸ ± 3.10 × 10⁸ cells/L), T3 (3.79 × 10⁸ ± 3.18 × 10⁷ cells/L), T1 (2.28 × 10⁸ ± 1.49 × 10⁷ cells/L), and T0 (4.5 × 10⁷ ± 6.06 × 10⁶ cells/L) with high significant difference (F(4, 84) = 5.35, p < 0.00). Mean densities obtained in treatments T2, T1, and T0 were not significantly different among them. In return, we observe significant difference between treatment T0 and others in the one hand and among treatments T4, T3, and T2, and T1 on the other hand (p < 0.05). Globally, in terms of total density, treatment can be classified as follows: T5 > T4 > T3 > T2 > T1 > T0.
Figure 3. Phytoplankton density evolution in the classes per treatment (dose of rabbit manure). Cos: Coscinodiscophyceae, Eug: Euglenophyceae, Bac: Bacillariophyceae, Con: Conjugatophyceae, Chl: Chlorophyceae. T0 (the control), T1, T2, T3, T4, T5 corresponding respectively to 0, 300, 600, 900, 1200, 1500 g of dry rabbit manure (RM).
Figure 4. Evolution of different phytoplankton phylum. T: treatments Cos: Coscinodiscophyceae, Eug: Euglenophyceae, Bac: Bacillariophyceae, Con: Conjugatophyceae, Chl: Chlorophyceae. T0 (the control), T1, T2, T3, T4, T5 corresponding respectively to 0, 300, 600, 900, 1200, 1500 g of dry rabbit manure (RM).

4. Discussion

Several studies focused on freshwater and marine phytoplankton showed the importance of environmental conditions [31–33]. Indeed, the mean temperature of 34 °C recorded in our culture media was higher than 25 °C and near 35 °C obtained respectively during the production of Isochrysis galbana [31] and Scenedesmus abundans [34]. According to the same authors, at these temperatures, algae are able to synthetize maximum quantity of glucides and lipids contrary to proteins that are optimally synthesized at lower temperature (15 °C). At this temperature, sun light allow algae to consume the CO2 released through bacterial mineralization of organic matter and to produce oxygen that is indispensabl e to aquatic living beings, as well as bacteria that use it in mineralization processes and ammonium oxidation [35,36]. These facts justify low ammonium rates and high nitrate concentrations observed in the current study (Figure 1). Thus, the main methods to eliminate the ammonium that come from organic matter (rabbit manure) could be volatilization and nitrification [29,37]. The pH value recorded in culture media varied from 6.85 to 7.16 and is near that recorded by Hodaifa et al. [38], which is 7 during the production of Scenedesmus obliquus. According to Hodaifa et al. [38], neutral pH media are favorable to maximal proteins and chlorophylls synthesis by algae. In return, pH values recorded indicate low photosynthetic activity of algae; this could be explained by the fact that it could be accumulated at the ampoule of buckets due to the absence of mixing and would not benefit from the available light. These observations are in accordance with those of Zimmo et al. [39] according to which, in waste water ponds, photosynthesis of algae was limited by the water column and then low pH values comprised 6.9–7.3 were observed. Concerning dissolved oxygen, the best concentrations were recorded in fertilized media (7–9 mg O2/L). Indeed, these high dissolved oxygen concentrations obtained in our culture media through algae
photosynthesis [40] average 34 °C confirm the link existing between algae photosynthetic activity and temperature. These results corroborate with those of Bouali et al. [29] who obtained the highest oxygen rate (8.15 mg O2/L) during the dry season. In addition, this author proves the existence of high correlation between the dry period and the increase of oxygen level. High electrical conductivities of fertilized media indicate high mineralization and testify their mineral salts richness [4,30]. It results from this analysis that the rabbit manure used has highly affected the water quality in the different freshwater algae culture media. Orthophosphate concentration from 0.06 to 2.82 mg P-PO4³⁻/L assimilable by algae recorded in culture media is near that observed by Toyub et al. [41] ranging from 1.5 to 2.5 mg P-PO4/L orthophosphates for which production of *Scenedesmus obliquus* is maximal. Temperature and nutrient concentrations (nitrogen and phosphorus) constitute key factors to modify the predominance of the different phytoplankton species during the culture period. The highest density obtained in Chlorophyceae could be due to the different concentrations of inorganic nitrogen in culture media that range from 0.8 to 4 mg N/L recommended by Boyd [42] and Schlumberger et al. [43] for good growth of this phytoplankton class. High orthophosphate concentrations (0.5–3.5 mg/L) recorded were higher than that recommended (0.2–0.5 mg/L of orthophosphate) by the same authors in fish ponds dominated by Chlorophyceae beyond which the trend could be in favor of other species with more affinity. The high transparency value of Secchi disk in treatment T0 contrary to the other fertilized treatments associated to the high orthophosphate concentration and pH inferior to 9 could forecast predominance of Chlorophyceae [43]. The highest of this latter was recorded in treatment T5 and could be the source of low density of Chlorophyceae to the profit of Bacillariophyceae (Coscinodiscophyceae). Barbe et al. [44] reported that ammonium and orthophosphates concentrations higher than those recommended as well as ratio P-PO4/N (inorganic nitrogen) superior to 1/8 and 1/10 could provoke development of Cyanobacteria even we notice their absence in our culture media. Indeed, this high phosphorus concentration could be due to the death of certain algae and bacteria in the culture medium and could constitute the second source of organic fertilizer. In addition, the highest phytoplankton biomass obtained in treatment T5 is tied to its phosphorus richness. Indeed, phosphorus constitutes limiting factor to phytoplankton production in aquatic medium [43]. However, the most assimilable fraction is orthophosphate [45] and often represents a small part of free phosphate in culture media [11,46,47]. Additionally, the highest algae density (7.91 × 10⁸ ± 6.78 × 10⁷ cells/L) and the highest mean rate of chlorophyll-a (14.47 mg/L) recorded in treatment T5 (1500g/m³) could be tied to trophic enrichment phenomenon in fertilized media that could lead to high organic load. Consequently, the use of treatment T5 could provoke eutrophication in fish production systems. Indeed, 14 mg/L of chlorophyll-a is the maximal value recommended by Agadjihouèdé et al. [11]. It would be judicious not to use this dose in fish farming ponds to avoid excessive development of certain toxic and indigestible Cyanobacteria. However, the high Cyanobacteria density known by its nutritional richness and digestibility, constitute a trump for optimization of zooplankton and micro-invertebrates. Mean chlorophyll-a concentrations obtained in treatments T2 (4.67mg/L), T3 (3.60 mg/L) and T4 (3.92 mg/L) were superior to the minimal value of 2 mg/L proposed by Canovas et al. [48] for good primary production. Thus, T2, T3, and T4 would offer very good nutritive conditions to algae. Algae densities obtained in T2, T3, and T4 were higher than those obtained by Toyub et al. [41] that are, respectively, 97.05; 83.21; 65.19, and 51.21 (∗10² cells/L) cultivated from soft drink factory effluents at different concentrations. By the same way, mean densities obtained in these studies are higher than those obtained by Sipaaiba-Tavares et al. [1] during the culture of *Ankistrodesmus gracilis* (144 × 10¹ cells/L) based on NPK. These differences could be explained by the nature of the substrates and culture media.

In addition, the analysis of phytoplankton classes in our culture media sets first Chlorophyceae, followed by Bacillariophyceae, Conjugatophyceae, Coscinodiscophyceae, and Euglenophyceae, which constitute predominant algae groups in fish farming ponds revealing nutrient availability. However, low densities of Euglenophyceae could be due to low organic load [17,27,29,49,50] but also consumption by zooplankton. Additionally, Chlorophyceae and Euglenophyceae are easily edible by many zooplankton, such as Cladocera, belonging to the *Daphnia* genus, as well as small
conflicts of interest. According to Barbe et al. [44] and Schlumberger et al. [43], Bacillariophyceae (Diatomeae) and Conjugatophyceae (Desmidials) are considered as secondary species according to nutrient concentrations in production medium and also present in oligotrophic water. This approach of algae production is being improved to generate high algae biomass that constitutes primary production not only for fish production but also for renewable energies [4,52,53]. Analysis of physico-chemical parameters, in general, and especially dissolved oxygen, conductivity, pH, and nutrients concentration (nitrogen and phosphorus) as well as the different densities of phytoplankton classes showed that genuine animal organic fertilizers (rabbit manure) are suitable for primary production favoring optimal fish farming at a cheap price.

5. Conclusions

The current study shows the valorization of rabbit manure through the production of multispecies freshwater algae for fish farming. It results from this study that treatment T2, T3, T4, and T5 respectively 600, 900, 1200, and 1500 (g/m³), provided good algae productions, while, treatment T5 shows a risk of eutrophication. In application, treatments T2, T3, and T4 could constitute rabbit manure doses to be used for massive production of multi-species freshwater phytoplankton easily edible by zooplankton and macro-invertebrates. Beyond the current quantitative evaluation, it is important to envisage in forthcoming studies the bromatological analysis of these algae in order to estimate their nutritive components.

Author Contributions: Conceptualization, A.R.; methodology, L.M.N.D.; software, D.G.; validation, A.D.C. and A.M.T.C.; formal analysis, M.J.C.; investigation, M.J.C.; resources, F.D.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We thank the Ministry of Higher Education and Scientific Research (MESRS) of Benin Republic that funded this work through the project “Training of Trainers of Benin Universities”.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sipaúba-Tavares, L.H.; Pereira, A.M.L. Large scale laboratory cultures of Ankistrodesmus gracilis (Reisch) Korsikov (Chlorophyta) and Diaphanosoma biergei Korinek, 1981 (Cladocera). Braz. J. Biol. 2008, 68, 875–83.
2. Hecht, T. A review of on-farm feed management practices for North African catfish (Clarias gariepinus) in sub-Saharan Africa. In On-Farm Feeding and Feed Management in Aquaculture; Hasan, M.R., New, M.B., Eds.; FAO Fisheries and Aquaculture Technical Paper: Rome, Italy, 2013; Volume 583, pp. 463–479.
3. Djissou, A.S.M.; Tossavi, E.C.; Vodounnou, J.D.; Toguyeni, A.; Fiogbe, E.D. Valorization of agro-alimentary waste for production of maggots like source of proteins in the animal feeds. Int. J. Agron. Agric. Res. 2015, 7, 42–46.
4. Adandé, R.; Bokossa, H.K.J.; Liady, M.N.D.; Fiogbe, E.D. Valorization of various sources of rabbit manure in agro-piscicultural system in Benin (West Africa): Dynamics and effect of mineralization upon quality of fresh water. Int. J. Recycl. Org. Agric. Waste 2017, 6, 233–243.
5. Saint-Jean, L.; Bonou, C.A.; Pagano, M. Développement et croissance en poinds de Moina (cf) micrura et de Mesocyclops agunnus dans un milieu saumâtre tropical: Les étangs de pisciculture de Layo (Côte d’Ivoire). Rev. Hydrobiol. Trop. 1994, 24, 287–303.
6. Dalme, D.K.; Chari, M.S. Performance evaluation of different animal wastes on culture of Daphnia Sp. J. Fish. Aquat. Sc. 2011, 6, 57–61, doi:10.3923/jfas.2011.57.61.
7. Gobler, C.J.; Renaghan, M.J.; Buck, N.J. Impacts of nutrients and grazing mortality on the abundance of Aureococcus anophagefferens during a New York brown tide bloom. Limnol. Limnol. Oceanogr. 2002, 47, 129–141.
8. Akodogbo, H.H.; Bonou, C.A.; Adande, R.; Sossou, D.S.; Fiogbe, E.D. Optimization of zooplankton production from pig dung optimal dose: Renewed medium. Agric. Adv. 2015, 4, 15–21, doi:10.14196/aa.v4i2.1804.
9. Adandé, R.; Liady, M.N.D.; Bokossa, H.K.J.; Djidohokpin, G.; Zouhir, F.; Mensah, G.A.; Fiogbe, E.D. Utilisation rationnelle de fertilisants organiques pour la production de macroinvertébrés benthiques d’eau douce en pisciculture. Biotechnol. Agron. Soc. Environ. 2018, 22, 12.
10. Sander, K.; Murthy, G.S. Life cycle analysis of algae biodiesel. Int. J. Life Cycle Assess 2010, 15, 704.
11. Agadjihouéédé, H.; Bonou, A.C.; Montchowui, E.; Laleyé, P. Recherche de la dose optimale de fiente de volaille pour la production spécifique de zooplancton à des fins piscicoles. Cah. Agric. 2011, 20, 247–60.
12. Arun, K.M.; Padmavati, G.; Anandavelu, I. Biochemical composition and calorific value of zooplancton from the coastal waters of South Andaman. Proceed. Int. Acad. Ecol. Environ. Sci. 2013, 3, 278–287.
13. Nandini, S.; Ortiz, A.R.N.; Sarma, S.S.S. Elaphoidella grandidiieri (Harpacticoidea: Copepoda): Demographic characteristics and possible use as live prey in aquaculture. J. Environ. Biol. 2011, 32, 505–511.
14. Adandé, R.; Adjahouinou, D.C.; Liady, M.N.D.; FIOGBE E.D. Alimentation des lapins (Oryctolagus cuniculus L.) à base de Azolla filiculoides, Elaès guineensis, Ipomoea aquatica et Panicum maximum: Effet sur la croissance des lapins et potentiel nutritif des crottés pour l’aquaculture. Int. J. Biol. Chem. Sci. 2017, 11, 2914–2923.
15. Santeiro, M.R.; Ricardo Motta, P.-C.; Sipaúba-Tavares, L.H.D. variation of zooplancton biochemical composition and biomass in plankton production tanks Acta Scientiarum. Biol. Sci. 2006, 28, 103–108.
16. Agadjihouéédé, H.; Bonou, C.A.; Chikou, A.; Laleyé, P. Production comparée de zooplancton en bassins fertilisés avec la fiente de volaille et la bouse de vache. Int. J. Biol. Chem. Sci. 2010, 4, 432–442, doi:10.4314/ijbcs.v4i2.58143.
17. Adjahouinou, D.C.; Liady, N.D.; Fiogbe, E.D. Diversité phytoplantonique et niveau de pollution des eaux du collecteur de Dantokpa (Cotonou-Bénin). Int. J. Biol. Chem. Sci. 2012, 6, 1938–1949.
18. Bourrelly, P. Les Algues d’Eau Douce: Algues Jaunes et Brun (Tome 2). Edition Boubée N et Cie: Paris, France, 1981, p. 521.
19. Bourrelly, P. Les Algues d’Eau Douce: Algues Bleues et Rouges (Tome 3); Edition Boubée N. et Cie: Paris, France, 1985, p. 608.
20. Bourrelly, P. Les Algues d’Eau Douce:Algues Vertes (Tome 1); Edition Boubée N. et Cie: Paris, France, 1990; p. 576.
21. Compère, P. Algues de la région du lac Tchad. II- Cyanophycées. Cah. Orstom Série Hydrobiol. 1974, 8, 165–198.
22. Compère, P. Algues de la région du lac Tchad. III- Rhodophycées, Euglenophycées, Cryptophycées, Dinophycées, Chrysophycées, Xanthophycées. Cah. Orstom. Série Hydrobiol. 1975, 9, 167–192.
23. Compère, P. Algues de la région du lac Tchad. IV- Diatomophycées. Cah. Orstom. Série Hydrobiol. 1975, 9, 203–290.
24. Compère, P. Algues de la région du lac Tchad. V- Charophycophytes 1ème partie. Cah. Orstom. Série Hydrobiol. 1976, 10, 77–118.
25. Compère, P. Algues de la région du lac Tchad. VI- Charophycophytes 2e partie: Ulotrichophycées, Zygomérateées. Cah. Orstom. Série Hydrobiol. 1976, 10, 135–164.
26. Compère, P. Algues de la région du lac Tchad. VII- Charophycophytes 3e partie: Desmiidées. Cah. Orstom. Série Hydrobiol. 1977, 11, 77–177.
27. Durand, J.R.; Lévêque, C. Flore et Faune Aquatiques de l’Afrique Sahelo- Soudanienne (Tome 1); Durand, J.R., Lévêque, C., Eds.; Editions ORSTOM, Collection Initiations Initiations-Documents Techniques. Paris: 1980; Volume 44, pp. 9–61.
28. Guiry, M.D. Algae Base. Worldwide Electronic Publication, National University of Ireland, Galway. Available online: http://www.algaebase.org (accessed on 25 June 2017).
29. Bouali, M.; Zrafi, I.; Mouna, F.A. Bakhrouf, Pilot study of constructed wetlands for tertiary wastewater treatment using duckweed and immobilized microalgae. Afr. J. Microbiol. Res. 2012, 31, 6066–6074.
30. Rodier, J.B.L.; Merlet, N. Coll. Analyse de l’eau; Paris: 2012; p. 20225.
31. Zhu, C.J.; Lee, Y.K.; Chao, T.M. Effects of temperature and growth phase on lipid and biochemical composition of Isochrysis galbana TK1. J. Appl. Phycol. 1997, 9, 451–457.
32. Vargas, S.; Leslie, K.; Vacek, P.; Socinski, M.; Weaver, D. Estrogen-receptor-related protein p29 in primary nonsmall cell lung carcinoma: Pathologic and prognostic correlations. Cancer Interdiscip. Int. J. Am. Cancer Soc. 1998, 82, 1495–1500.
33. Liady, M.N.D.; Kpèhouénou, O.B.; Adandé, R.; Nounavo, A.D.P.; Kouadio, L.A.; Aina, M.P.; Fiogbe, E.D. Valorisation of the supernatant of brewery effluent in plankton production for fish farming: An alternative for environment protection in southern countries. *Biotec. Agron. Soc. Environ.*, 2020, 24 (4), 235–239.

34. Tahiri, M.; Benider, A.; Belkoura, M.; Dauta, A. Caractérisation biochimiques de l’algue verte *Scenedesmus abundans*: Influence des conditions de culture. In *Annales de Limnologie-International Journal of Limnology*; EDP Sciences: Ulis, France, 2000.

35. Oswald, W.J. Micro-algae and wastewater treatment. In *Micro-algal Biotech*; Bor-owitza, M.A., Borowitza, L.J., Eds.; Cambridge University Press: Cambridge, UK, 1988; pp. 305–328.

36. Muñoz, R.; Guieysse, B. Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Res.* 2006, 40, 2799–2815.

37. Shen, G.; Xu, J.; Hu, S.; Zhao, Q.; Liu, Y. Nitrogen removal pathways in shallow-water duckweed-based wastewater treatment systems. *J. Ecol. Rural Environ.* 2006, 22, 42–47.

38. Hodaifa, G.M.; Martínez, E.; Sánchez, S. Influence of pH on the Culture of *Scenedesmus obliquus* in Olive-mill Wastewater. *Biotechnol. Bioprocess Eng.* 2009, 14, 854–860.

39. Zimmo, O.R.; Van, D.N.P.; Gijzen, H.J. Nitrogen mass balance across pilot-scale algae and duckweed-based wastewater stabilisation ponds. *Water Res.* 2004, 38, 913–920.

40. Hamaidi, M.S.; Hamaidi, F.; Zoubiri, A.; Benouaklil, F.; Dhan, Y. Etude de la dynamique des populations phytoplanctoniques et résultats préliminaires sur les blooms toxiques a cyanobactéries dans le barrage de Grib (Ain Defla-Algérie). *Europ. J. Sci. Res.* 2009, 32, 369–380.

41. Toyub, M.A.; Miah, M.I.; Habib, M.A.B.; Rahman, M.M. Growth performance and nutritional value of *Scenedesmus obliquus* cultured in different concentrations of sweetmeat factory waste media Bang. *J. Anim. Sci.* 2008, 37, 86–93.

42. Boyd, C.E. *Water Quality Management for Pond Fish Culture*; Elsevier Scientific Publishing Co.: Amsterdam, The Netherlands, 1982; p. 318.

43. Schlummerger, O.; Bouretz, N. Réseaux trophiques et production piscicole en étangs fertilisés (Dordogne, France). *J. Water Sci.* 2002, 15, 177–92.

44. Barbe, J.; Schlummerger, O.; Bouretz, N. Evaluation de la production piscicole potentielle des étangs. *IRSTEA*, édition 2000, p. 49–62, hal-00464073.

45. Reynolds, C.S. The ecology of freshwater phytoplankton. In *Cambridge Studies in Ecology*; Beck, E., Ed.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 1984.

46. Moussa, M.; Baccar, L.; Ben, K.R. La lagune de Ghar El Melh: Diagnostic écologique et perspectives d’aménagement hydraulique. *J. Water Sci.* 2005, 18, 13–26.

47. Benzha, F.; Taoufik, M.; Dafir, J.E.; Kemmou, S.; Loukili, L. Qualité physico-chimique des eaux du réservoir Daourat; impact de la vidange sur son fonctionnement. *J. Water Sci.* 2005, 18, 57–74.

48. Canovas, S.; Casellas, C.; Picot, B.; Pena, G.; Bontoux, J. Evolution annuelle du peuplement zooplanctonique dans un lagunage à haut rendement et incidence du temps de séjour. *J. Water Sci.* 1991, 4, 69–89.

49. Gonzalez, C.; Marciniak, J.; Villaverde, S.; Garcia-Encina, P.A.; Munoz, R. Microalgae processes for the degradation of pre-treated piggery wastewaters. *Appl. Microbiol. Biotech.* 2008, 80, 891–898.

50. Ignacio, G.; Virginia, A.V.; Saul, B.; Maria, C.; Roberto, S.; Pedro, A.; Eloy, B.; Raul, M.A. Comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. *Bioresour. Technol.* 2010, 101, 5150–5158.

51. Pourriot, R.; Meybeck, M. *Limnologie Générale*; Masson: Paris, France, 1995.

52. Rawat, I.; Ranjith, K.R.; Mutanda, T.; Bux, F. Dual role of microalgae: Phycoremidation of domestic wastewater and biomass for sustainable biofuels production. *Appl. Energ.* 2011, 88, 3411–3424.

53. Rusten, B.; Sahu, A.K. Microalgae growth for nutrient recovery from sludge liquor and production of renewable bioenergy. *Water Sci. Technol.* 2011, 64, 1195–1201.

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.