Journal of Fisheries and Aquaculture
ISSN: 0976-9927 & E-ISSN: 0976-9935, Volume 3, Issue 2, 2012, pp.-47-51.
Available online at http://www.bioinfo.in/contents.php?id=68

WATER QUALITY AND SEDIMENT FEATURES IN PONDS WITH NILE TILAPIA (*Oreochromis niloticus* L.) FED *AZOLLA*

ABOU YOUSSOUF
Unité de Recherche sur les Zones Humides, Faculté des Sciences et Techniques, Département de Zoologie, Université d'Abomey-Calavi, BP 526 Cotonou, Bénin.
*Corresponding Author: Email- y_abou@yahoo.com

Received: September 05, 2012; Accepted: October 01, 2012

Abstract- The effects of feeding Nile tilapia with *Azolla*, an atmospheric nitrogen fixing fern, on water quality and sediments characteristics was evaluated in 90 days pond experiment. Fish were fed with six diets differing by their level in the *Azolla* meal (AM), as partial fishmeal (FM) substitutes: 0%, 10%, 20%, 30%, 40% and 50%. The *Azolla*-free diet (0%) served as a control.

Growth performances were higher in fish fed the control diet and a significant decreasing trend was obtained in fish fed diets containing more than 20% of AM ($P<0.05$). Significant higher concentration in soluble reactive phosphate (SRP)(0.14-0.38mg l^{-1}) and total phosphorus (TP) (0.63-1.22mg l^{-1}) were found in water of ponds with 0 and 10% AM ($P<0.05$). P content of soil matter (0.01-0.02%) was identical in all ponds ($P>0.05$). Values of nitrogen compounds in water were similar in all experimental ponds. The sediment C/N atomic ratios ranged from 6.7 to 19.6, indicating an influence of allochthonous material in ponds. This C/N ratio which decreased significantly in high AM-ponds ($P<0.05$) was directly influenced by the AM-nitrogen provided: $C/N\ at. = -0.0057N^2+0.0303N+18.238$ ($R^2 = 0.79$; $P<0.001$).

The present study proved that the fern *Azolla* could sustain Nile tilapia growth by serving not only as feed ingredient but as a nitrogen providing source in rearing ponds, thus improving water natural productivity, beneficially to phytoplanktonophagous fish in pond aquaculture.

Keywords- *Azolla*, Fish meal replaces, *Oreochromis niloticus*, sediment, atomic ratio C/N.

Citation: Abou Youssouf (2012) Water Quality and Sediment Features in Ponds with Nile Tilapia (*Oreochromis niloticus* L.) Fed *Azolla*. Journal of Fisheries and Aquaculture, ISSN: 0976-9927 & E-ISSN: 0976-9935, Volume 3, Issue 2, pp.-47-51.

Introduction

The intensification of aquaculture leads to the release of organic wastes and inorganic nutrients that promote environmental pollution [1], thus compromising the sustainability and expansion of the enterprise. The nitrogen and phosphorus nutrients in cause originate mainly from fish feeds and thus fishmeal-based-diets are considered as primary pollutants of aquatic ecosystems. Indeed, several studies have reported that the high P of fish meal-based diets is not well utilized by many fish species, as reported with common carp *Cyprinus carpio* L., gibel carp *Carassius auratus gibelio* or rainbow trout *Onchorhynchus mykiss* [2-4]. Therefore, the better way is to balance this nutrient in feeds for farmed species. Plants protein that contains less Phosphorus with high availability could help to achieve this goal [5]. Of these plants, the aquatic fern *Azolla* has been successfully used recently in tilapia aquaculture as feed ingredients [6-11] and as environmentally-friendly ingredient to prevent eutrophication in ponds [9,12]. This small floating freshwater fern develop a symbiotic relationship with a cyanobacteria named *Anabaena azollae* Strasburger, an atmospheric nitrogen fixing organism. Due to its presence in *Azolla* fronds cavity, A. *azollae* make the fern naturally rich in nitrogen. This property is exploited in rice-field culture where *Azolla* used solely is known as biofertilizer [13]. In semi-intensive aquaculture, the main ecological processes occurring in ponds concern the accumulation of organic matter (uneaten feed), senescent phytoplankton and fish faeces and the decomposition and mineralization processes that transform the material into inorganic forms. The contribution of each source to organic matter concentration in soil is very difficult to quantify. However, Boyd and Tucker [14] noticed that 15% of feed sediments as uneaten feeds, whereas 30% of the ingested feed is excreted [15]. Phytoplankton is considered the major source of organic matter [16], whereas the main sources of organic nitrogen should include the uneaten feeds and faecal wastes released as soluble and solid forms into water. Faeces generally account for 5-15% of the nitrogen originally ingested by fish and 25-80% is excreted as ammonia or dissolved organic nitrogen [15,17], indicating that nitrogen released into fish ponds should depend on the quantity and quality of nitrogen in feeds, as well as on nutrient utilization by the fish. So far, research on *Azolla* in fish farming has strongly focused on its effects on growth performance, fish carcass composition and quality [10,11] and the effects of feeding with *Azolla*-diets on pond water quality are very scarcely evoked.

The present study aimed at evaluating water quality and pond sediment characteristics in response to feeding Nile tilapia with *Azolla*-diets in stagnant earthen ponds; the final objective being to better understand the intrinsic role of the fern *Azolla* in pond aquaculture, especially in tilapia fish farming.

Material and Methods

Experimental Design and Set-up

The experiment was carried out during 90 days in newly eighteen small ponds of $30m^2$ (10m x 3m x 1m) filled naturally from the water table and stocked with male Nile tilapia *O. niloticus* (initial mean weight= 16.3 ± 0.1 g) at a density of $2m^{-2}$ (60 fish $pond^{-1}$).

These ponds were randomly assigned to 6 triplicate (6 x 3) groups, each set attributed one of the experimental diets. Diets are isonitrogenous (29.2% crude protein) and isoenergetic (16.9 kJ g^{-1}), formulated using locally available ingredients and the freshwater fern *Azolla filiculoides* Lamarck. Diets were formulated to contain 0% (A_0), 10% (A_{10}), 20% (A_{20}), 30% (A_{30}), 40% (A_{40}) and 50% (A_{50}) of *Azolla* meal (AM). The *Azolla*-diets were compared with a control (A_0) without AM.

Formulation and proximate composition of experimental diets are given in [Table-1]. Diets were prepared according to the procedure described in Abou, et al. [7] and preserved in the refrigerator (+4° C) until used for feeding fish.

Fish were hand-fed daily according to Melard [18]. Daily rations were divided into two parts, each distributed at 8:00 hrs. and 16:00 hrs. respectively.

Once every fortnight, at least 40% of the fish in stock were sampled with a dip net, without entering the pond and weighed. The daily ration was adjusted fortnightly according to the actual body weight.

Limnological Evaluation of Water and Sediment

Water quality was monitored fortnightly. Water temperature and dissolved oxygen (using an oxythermometer WTW Oxi 197i, WTW, Weilheim, Germany, precisions: ± 0.01°C and ± 0.01mg L^{-1}) and pH (using a pH meter WTW pH 330, precision: ± 0.01) were measured at 10cm depth at the following times: 8:00, 11:00, 14:00 and 17:00 hrs. Water transparency was measured using a Secchi disk. Nitrate, nitrite, ammonium and soluble reactive phosphate levels were determined by cadmium reduction, sulfanilamide, phenate and ascorbic acid methods respectively, according to APHA [19].

Estimates of chlorophyll *a* [20] and zooplankton abundance (by counting concentrated samples obtained after filtering 20 L of water from three points in each pond through a 55-µm plankton net) were also carried out.

Sediment samples were collected before fish stocking and at the end of the experiment. Samples were randomlytaken in five spots from the upper 5cm stratum using a core sampler [14]. The samples were mixed to form a composite sample from which subsamples were drawn for analysis. Organic matter was measured after incineration at 550°C for 24 hrs. Total carbon and total nitrogen in the sediment were determined using a NA 1500 Nitrogen/Carbon Analyzer (Carlo Erba). Then, the atomic C/N ratios were evaluated. The persulphate digestion methods [21] followed by the ascorbic acid method was used to determine the total phosphorus content in soil.

Table 1- Formulation and proximate composition (on dry matter basis) of experimental diets

Diets	A_0	A_{10}	A_{20}	A_{30}	A_{40}	A_{50}
Ingredients (g/100 g diet)						
Fish meal	30	25	20	15	10	5
Azolla meal	0	10	20	30	40	50
Cottonseed meal	30	30	30	30	30	30
Maize bran	18	17	16	15	14	10
Brewery draff	20	16	12	8	4	3
Cassava starch*	1	1	1	1	1	1
Salt (NaCl)	1	1	1	1	1	1
Proximate Composition						
Dry matter (%)	90.3	90.2	91.1	89.4	90.5	90.3
Crude protein (%)	29.3	29.3	29.2	29.1	29.2	29
Crude lipid (%)	10.8	10	9.4	9	8.4	8
Crude ash (%)	14.3	13.8	12.5	12.2	10.9	10
Crude fibre§ (%)	9.9	10	10.2	10.4	10.5	10.8
NFE† (%)	35.7	36.8	38.7	39.4	41	42.2
Gross energy‡ (kJ/g)	17	16.9	16.9	16.9	17	17
Phosphorus (%)	1.13	1.08	1.06	1	0.95	0.76

**Binder*

§Calculated according to Ovograin Feeds Depot, Abomey-Calavi, Bénin and Leonard [22] for ingredients and Azolla filiculoides, respectively.

†Nitrogen-Free Extract, calculated as: 100-(%protein + %lipid + % ash + %crude fibre)

‡Calculated according to Tacon [23].

Statistical Analysis

Means for growth performances, water and sediment quality parameters were analyzed using one-way Anova, after the verification of the homogeneity of their variance [24]. When the effect was significant, comparisons between treatment means was run using Duncan's multiple range test [25] at P=0.05.

Results

Among all the treatments, fish survival rate ranged did not show any significant differences and values were higher than 85%. As shown in [Fig-1], fish final mean weight decreased from fish fed the control diet to those fed with 50% AM, values ranging from 88.8 g to 135.9 g.

Mean values of fortnightly measurements of transparency (range: 35.3-38.9cm), temperature (range: 29.8-30.2°C), pH (range: 6.33-6.62), dissolved oxygen (range: 4.65-5.49mg l^{-1}), nitrite (range: 0.01-0.02mg l^{-1}), nitrate (range: 0.22-0.32mg l^{-1}), ammonium (range: 0.28-0.43mg l^{-1}), total nitrogen (range: 0.70-0.79mg l^{-1}), chlorophyll *a* (range: 16.8-18.7µg Chl*a* l^{-1}) and zooplankton abundance (range: 759-862 ind.l^{-1}) did not show significant differences between triplicates (P>0.05).

Significant variations in SRP (range: 0.14-0.38mg l^{-1}) and total phosphorus (range: 0.63-1.22mg l^{-1}) were found (P<0.05) [Table -2], with higher concentrations in ponds A_0 and A_{10} than in other treatments. The P content of soil matter (range: 0.01-0.02%) was identical in all ponds ($P>0.05$). Lower values of soil organic matter were observed in ponds receiving A_{40} and A_{50} in comparison with those receiving A_0, A_{10}, A_{20} ($P<0.05$) [Fig-2]. As shown in [Fig-3], the C/N ratios of food were quite similar (range: 8.9-10.8) while the soil C/N atomic ratios (range: 6.7-19.6) decreased significantly, with lower values in ponds receiving high AM ($P<0.05$).

Nitrogen from *Azolla* in diets provided to fish per group of ponds was plotted with the soil C/N ratio of each pond [Fig-4]. The C/N ratio was directly influenced by AM-nitrogen provided (R^2= 0.79; $P<0.001$).

Table 2- Water quality parameters results in ponds with Oreochromis niloticus fed diets containing increasing levels of Azolla for 90 days. (A_0 to A_{50}: diet with 0% to 50% Azolla).

Diets	A_0	A_{10}	A_{20}	A_{30}	A_{40}	A_{50}
Transparency (cm)	35.3 ± 1.5	36.5 ± 3.3	37.2 ± 6.7	35.6 ± 2.5	38.3 ± 4.3	38.9 ± 5.3
Temperature (°C)	30.1 ± 0.2	29.8 ± 0.3	30.0 ± 0.3	30.2 ± 0.1	30.1 ± 0.2	30.2 ± 0.1
pH	6.62 ± 0.42	6.55 ± 0.22	6.47 ± 0.29	6.59 ± 0.2	6.43 ± 0.40	6.33 ± 0.45
Dissolved oxygen (mg l^{-1})	5.49 ± 0.38	5.31 ± 0.18	5.36 ± 0.55	5.28 ± 0.54	5.12 ± 0.63	4.65 ± 0.40
Nitrate mg l^{-1})	0.23 ± 0.04	0.22 ± 0.03	0.22 ± 0.05	0.28 ± 0.05	0.27 ± 0.07	0.32 ± 0.10
Nitrite (mg l^{-1})	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00
Ammonium(mg l^{-1})	0.28 ± 0.08	0.33 ± 0.02	0.34 ± 0.10	0.37 ± 0.10	0.43 ± 0.14	0.42 ± 0.13
Total nitrogen (mg l^{-1})	0.72 ± 0.04	0.70 ± 0.07	0.74 ± 0.03	0.79 ± 0.05	0.78 ± 0.04	0.77 ± 0.07
SRP (mg l^{-1})	0.38 ± 0.07^a	0.35 ± 0.02^a	0.29 ± 0.02^b	0.25 ± 0.02^{bc}	0.22 ± 0.03^c	0.14 ± 0.03^d
Total phosphorus (mg l^{-1})	1.22 ± 0.04^a	0.98 ± 0.09^b	0.89 ± 0.09^b	0.78 ± 0.05^c	0.70 ± 0.08^c	0.63 ± 0.04^c
Chlorophyll a (µg Chl a l^{-1})	18.1 ± 1.8	18.5 ± 0.9	17.9 ± 2.1	18.7 ± 1.7	17.7 ± 1.3	16.8 ± 0.4
Zooplankton (no. l^{-1})	836 ± 80	759 ± 71	774 ± 48	802 ± 30	862 ± 96	805 ± 48
P content of soil matter (% DM)	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00

^{a,b,c,d} Means with no letters or with same letters as superscripts are not significantly different ($P > 0.05$). Data are means of three replicates. DM means dry matter.

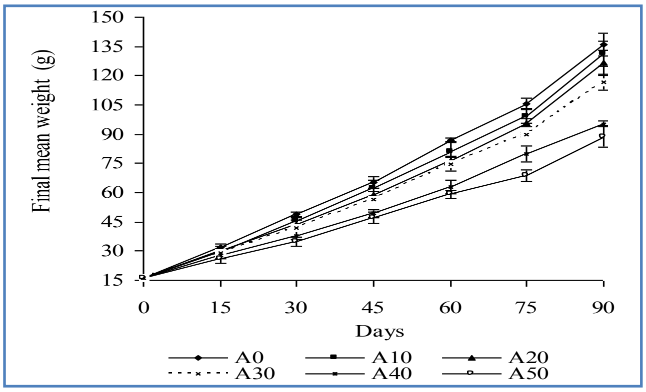

Fig. 1- Changes in fortnight mean weight (mean ± S.D.) of Nile tilapia fed in earthen ponds for 90 days with diets containing increasing level of *Azolla filiculoides*. A_0 to A_{50}: diet with 0% to 50% *Azolla*.

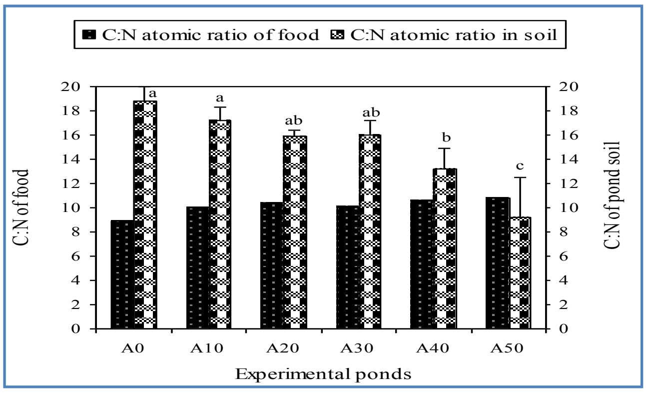

Fig. 2- Soil organic matter and total food supplied after 90 days of experiment in ponds with Nile tilapia fed gradual level of *Azolla filiculoides*. For each parameter, bars bearing identical letters are not significantly different ($P>0.05$).

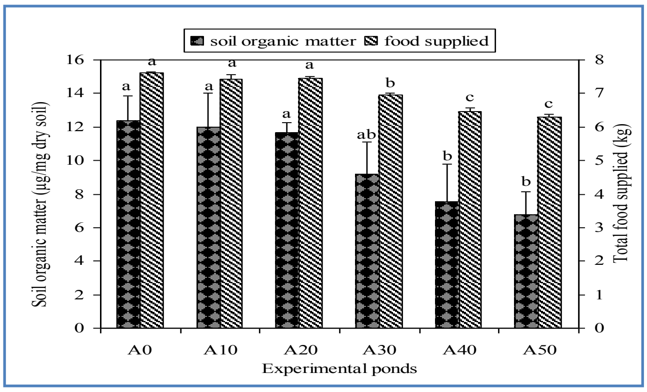

Fig. 3- C/N atomic ratios of food and in soil after 90 days of experiment in ponds of Nile tilapia fed with gradual level of *Azolla filiculoides*. Bars bearing identical letters are not significantly different ($P>0.05$).

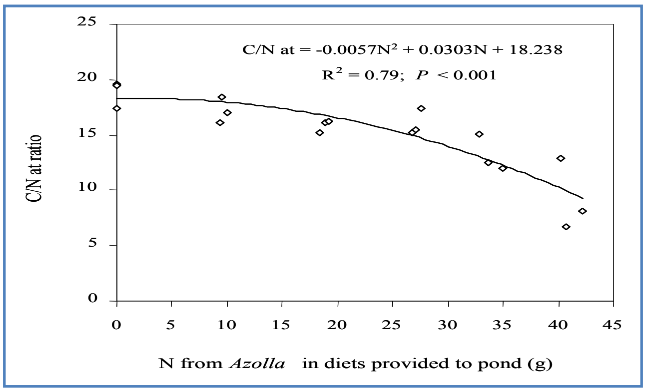

Fig. 4- Correlation between atomic C/N ratio and the contribution of *Azolla* in each diet to the total nitrogen supplied in each group of ponds.

Discussion

As shown in [Fig-1], the final mean weight decreased as *Azolla* mean increased in the experimental diets. These trends in growth variation were similar to those reported with other terrestrial and aquatic plants, as substitutes for FM. According to several authors, factors that limit the use of macrophytes in fish diets are the presence of antinutrient factors, or a deficiency in aminoacids and phosphorus. In this study, all the diets used contain sufficient amounts of phosphorus to meet the need of Nile tilapia [26,27]. The required aminoacids and minerals needed to balance the dietary deficiency at high content in AM could be likely met from pond ecosystem through natural productivity and water [28]. Thus, since *A. filiculoides* showed relatively low fibre content, and no antinutrient factors have been detected in that fern, the reduction observed in growth could probably be due to the lower protein digestibility of this fern, as mentioned by Leonard, et al. [29] in *Oreochromis aureus* Steindachner and Micha and Leonard [30] in *O. niloticus*.

This leads to a discharge of dissolved and solid P or N wastes resulting from a given quantity of feed. It seems important to analyze the dynamics of the resulting organic matter that, in combination with the non-negligible amount of uneaten feed, settled in sediment. We observed lower organic matter in soils from ponds A_{40} and A_{50} [Fig-2]. As the amount of food provided to each pond was based on fish growth in this study, the amount of food supplied to ponds A_{30}, A_{40} and A_{50} were lower, as the growth rates recorded in those pond were lower. Assuming that uneaten food sediments at the same rate, we may explain the lower organic matter in ponds A_{40} and A_{50} by the lower food supplied. The soil C/N ratio closely decreased with increasing AM in diets, i.e. the lower values for soil C/N ratios were recorded in ponds receiving high AM-diets. The C/N ratios of experimental diets were quite similar (8.9-10.8) and the nitrogen contributed by the *Azolla* fern in diets increased with AM proportion, reaching 49% of the total nitrogen in diets A_{50}. As the C/N ratio of soil is positively related to the decomposition rate [30], the lower C/N ratio values in ponds receiving higher AM implies a lower decomposition rate and so an accumulation of organic matter in those ponds. According to Jiménez-Montealegre, et al. [31], the soil C/N ratio is largely dependent on nitrogen, as organic nitrogen decomposition is more rapid than organic carbon. Since autochthonous sources in ponds, i.e., plankton and bacteria, have low C/N atomic ratio, ranging from 5 to 8 [32], the values of soil C/N ratio measured, which ranged from 6.7 to 19.6, implies an influence of organic matter from exogenous sources.

As shown in [Fig-3], C/N ratios decreased with increasing the amount of AM, to get close to the values of C/N ratios in food. So, the lower C/N ratio in ponds receiving high AM implies a lower decomposition rate of AM-nitrogen and then its accumulation in ponds. On the other hand, higher amount of organic matter and higher decomposition rate (higher soil C/N ratio) were recorded in ponds receiving A_0, A_{10}, A_{20}. This may explain the higher values of SRP and total P in water of ponds A_0 and A_{10}. Despite the differences in nitrogen and phosphorus compounds in experimental ponds, the phytoplankton biomass measured as Chlorophyll *a* concentrations did not vary between treatments.

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