



Evaluation of nitrogen and phosphorus wastes produced by Nile tilapia (*Oreochromis niloticus* L.) fed *Azolla*-diets in concrete tanks

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ABSTRACT

Nitrogenous (N) and phosphorus (P) wastes discharged into water by Nile tilapia *Oreochromis niloticus* L. (initial mean weight: 16.4 g) fed in tanks for 90 days was studied. Fish were fed with six isonitrogenous (29.2% crude protein) and isoenergetic (16.9 KJ g⁻¹) diets A₀, A₁₀, A₂₀, A₃₀, A₄₀ and A₅₀, containing 0%, 10%, 20%, 30%, 40% and 50% of *Azolla* meal (AM) respectively, as partial fishmeal (FM) substitutes. Diet A₀, without AM, served as a control. Growth performances were higher with the control diet, and a decreasing trend was found when AM level was higher than 10% in diets ($P < 0.05$). Identical values were recorded for crude protein and P content in fish. The total P (TP) discharged as waste show identical values (89.6-91.2 % supplied) for all experimental fish; while total N (TN) discharged (69.5%-80.7% supplied) increased significantly when AM level was greater than 20% in diets ($P < 0.05$). This study indicate that AM could be used up to 10% in diets without adverse effects on growth performances. High AM in diets affects growth but high amounts of undigested N from AM-protein is wasted and provided to water. However, the results suggest further investigations on the use of AM in diets that sustain growth and reduce P waste for eutrophication alleviation.

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Keywords: *Azolla*, fish meal replacement, *Oreochromis niloticus*, nitrogen, phosphorus, nutrient balance.

INTRODUCTION

The rapid growth in world aquaculture production over the past few decades has resulted in a concomitant increase in demand for fish meal (FM). Because of the stable

production of that feed ingredient at around 6.5 million tonnes, Hardy (2000) estimates that the amount required would be approximately 1.3 million tonnes less than 2.8 million tonnes required for aquaculture in

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2010. Limited availability of FM will subsequently represent a constraint for the growth of aquaculture production. Therefore, it is necessary to seek acceptable substitutes for this important ingredient. Another problem that could limit its sustainability is the negative impact of aquaculture on the environment due to FM. Indeed, the intensification of production results in the release of organic wastes and inorganic nutrients, such as phosphorus (P) and nitrogen (N), which are known to enrich and promote eutrophication in aquatic ecosystems (GESAMP, 1996). Therefore, environmental pollution associated with aquaculture practices becomes a critical issue for sustainability and expansion of this enterprise. In aquaculture, both P and N elements originate mainly from fish feeds (Cho and Bureau, 1997), due to their high levels in FM. Limited utilization of dietary P is often reported in fish lacking a stomach, such as common carp *Cyprinus carpio* L. and gibel carp *Carassius auratus gibelio* (Ogino et al., 1979; Zhang et al., 2006) in which high amount of unavailable dietary P is discharged as particulate matter in faeces (Steffens, 1989). Similarly, the excessive available dietary P can also be rapidly eliminated in dissolved form, probably through the gills and kidneys, as Nakashima and Leggett (1980) reported in rainbow trout *Onchorhynchus mykiss* Walbaum. Therefore, one should balance appropriately this nutrient in feed for farmed species. Whatever the physiological response of reared fish to dietary P, one of the numerous ways to reduce P waste produced by aquaculture is the use of FM substitutes that contain less P with high bioavailable proportion (Cho and Bureau, 2001). Among the most popular alternative ingredients used as FM substitutes, plant proteins are found to be worthwhile to meet this goal. The importance of the use of vegetable sources as FM substitutes is widely demonstrated in many fish growth studies (Fasakin et al., 2001; Afuang et al., 2003; Fiogbé et al.,

2004). But their role in developing low-polluting feeds by reducing P waste by fish is scarcely evoked. In that area, successful results had been reported by Jahan et al. (2003) in common carp and Nakashima and Leggett (1980) in rainbow trout. Also, a reduced P discharge has been demonstrated in fish fed diets in which FM was replaced by corn gluten meal (Kaushik et al., 2004). Of the various aquatic macrophytes that have been used with success in fish feeding, the freshwater fern *Azolla* can be cited (Leonard et al., 1998; Fasakin et al., 2001; Fiogbé et al., 2004). P content in *Azolla* species is lower than 0.77% dry matter (Leonard, 1997), which is by far lower to the level (2-4%) generally found in FM. This may encourage testing *Azolla* in developing low-polluting feeds. So far, *Azolla* research in fish farming is strongly focused on growth performance and digestibility in *O. niloticus* and *Oreochromis aureus* Steindachner (Leonard, 1997; Leonard et al., 1998; Micha and Leonard, 2001-2). Studies on its use as an "environmentally-friendly" ingredient are very scarce.

Therefore, the main purpose of this study was to investigate P and N wastes discharged into water by Nile tilapia *O. niloticus* fed with diets containing gradual levels of AM as FM partial substitutes.

MATERIALS AND METHODS

Fish, diets and feeding regimes

The experiment was carried out during 90 days in station (6°25'1.53"N 2°20'42.2"E) located at the University of Abomey-Calavi, Benin (West Africa). Male Nile tilapia *O. niloticus* (initial mean weight = 16.4 g) from a same cohort was bought from Lassissi fish farming centre (Porto-Novo, Benin). Sixty male fingerlings were stocked in each of eighteen 600 l square concrete tanks. These tanks were exposed to natural conditions (32-35°C; 12-h:12-h dark diurnal photoperiod) and were subjected to an enclosed recirculating system with a water flow rate of 2 l min⁻¹. They were randomly assigned to six

groups, each in three replicates (6 x 3 tanks), attributed to one of the diets.

Experimental diets were six isonitrogenous (29.2% crude protein) and isoenergetic (16.9 kJ g⁻¹), formulated to contain 0% (diet A₀), 10% (diet A₁₀), 20% (diet A₂₀), 30% (diet A₃₀), 40% (diet A₄₀) and 50% (diet A₅₀) of AM using locally available ingredients and the freshwater fern *Azolla filiculoides* Lamarck. The control diet A₀ is a practical diet used for production of marketed-size tilapia in Songhai centre (Porto-Novo, Benin). Formulation and proximate biochemical composition of experimental diets are given in Table 1. One-half liter of hot water with dissolved binder (cassava starch, obtained by cassava processing and bought in local market) was added to one kilogram of the diet thus formulated and mixed. The dough obtained was cut into paste and sun-dried for about three days at 32-35 °C. After drying, the diet was broken into small particles (5 mm) and preserved in refrigerator (+ 4°C) until used for feeding fish.

Fish were fed every day according to Melard (1986). Daily rations were divided into two parts, each hand-distributed at 8:00 h and 16:00 h, respectively. They were adjusted every two weeks according to the fish biomass in each tank.

Biochemical analysis

At the beginning of the experiment, thirty fish from the initial batch and six fish per tank at the end were randomly taken and mashed using a Robot coupe food processor. Subsamples were taken and stored at -20°C for carcass analysis. The diets used were also preserved at -20°C for biochemical analysis. Diets and whole fish were analyzed for dry matter (AOAC, 1990), crude protein (Nitrogen x 6.25, Kjeldahl method), crude fat (Folch et al., 1957) and crude ash content (incineration at 550°C in a furnace). Carcass N and P content in *Azolla* and diets were

determined by persulphate digestion (Gross and Boyd, 1998) with boric acid and sodium hydroxide.

Calculations

Growth and feed performance parameters were calculated as follow:

Final mean weight (W_f, in g) = FB (g) / number of fish harvested

Growth rate (g day⁻¹) = (W_f - W_i) day⁻¹

Specific growth rate (SGR, % day⁻¹) = 100 [ln(W_f) - ln(W_i)] day⁻¹

Food conversion ratio (FCR) = TFI x (FB - IB)⁻¹

Protein efficiency ratio (PER) = (FB - IB) DPI⁻¹

Apparent protein utilization (APU, %) = 100 x TPG x DPI⁻¹

where W_i and W_f are initial and final mean wet weight in g; TFI the total feed intake; FB and FI are final and initial fish biomass in g; DPI the total dietary protein intake; TPG the total body protein gain.

Apparent retention rate (%) of P and N, and the amount of P and N retained and discharged into water as wastes (faecal + urinary + gill) by fish were evaluated according to Cho et al. (1994) as follow:

Apparent retention rate (ARR, %) of P and N = 100 x (TN_f - TN_i) N_{supplied}⁻¹ (g),

Nutrient_{supplied} (NS, g) = N_{feed} x TFI

Nutrient_{retained} (NR, g) = NS x ARR x 100⁻¹

Total nutrient waste (TN or TP, g) = NS - NR

Where: TN_f and TN_i are the final and initial nutrient (P or N) body content in (g); N_{supplied} the quantity in (g) of nutrient (P or N) content in the total food supplied; N_{feed} is the quantity of feed supplied.

During the experiment, very limited feed loss was observed. Since this amount was unknown, we assume for calculations that the amount lost is 0.

Table 1: Formulation and proximate composition of experimental diets.

Diets	A ₀	A ₁₀	A ₂₀	A ₃₀	A ₄₀	A ₅₀
<i>Ingredients (g 100g⁻¹)</i>						
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
Binder*	1	1	1	1	1	1
Salt (NaCl)	1	1	1	1	1	1
<i>Proximate biochemical composition[‡]</i>						
Dry matter (%)	90.3	90.2	91.1	89.4	90.5	90.3
Crude protein (% DM)	29.3	29.3	29.2	29.1	29.2	29.0
Crude lipid (% DM)	10.8	10.0	9.4	9.0	8.4	8.0
Crude ash (% DM)	14.3	13.8	12.5	12.2	10.9	10.0
Crude fibre [§] (% DM)	9.9	10.0	10.2	10.4	10.5	10.8
NFE [†] (% DM)	35.7	36.8	38.7	39.4	41.0	42.2
Gross energy [‡] (kJ g ⁻¹)	17.0	16.9	16.9	16.9	17.0	17.0
Phosphorus (% DM)	1.13	1.08	1.06	1.00	0.95	0.76

*Cassava starch, obtained after cassava processing and bought in local market

[‡]Means of two analyses values

[§]Calculated according to Ovograin Feeds Depot, Abomey-Calavi, Bénin and Leonard (1997) for ingredients and *A. filiculoides*, respectively.

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

[‡]According to Tacon (1990)

DM: dry matter.

Statistical analysis

Data for growth, carcass composition and nutrient waste evaluation were analyzed using one-way analysis of variance (ANOVA). Differences between means were determined by Duncan's Multiple Range test (Duncan, 1955) to identify the differences among the triplicate groups at $P = 0.05$. Before analysis, homogeneity of variance was checked using the "Hartley test" (Hartley, 1959). Ratios and percentage data were log-transformed. All analysis were done using SPSS program version 13.0 (SPSS, Chicago, Illinois, USA).

RESULTS

Survival, growth rate and feed utilization

As shown in Table 2, survival rate (range: 89.04%-93.3%) did not show any significant differences and values were higher

in each treatment ($P > 0.05$). Fish growth rate decreased significantly when AM level exceeded 10% in the diet ($P < 0.05$). No significant differences were found in growth rate (range: 0.38-0.80 g day⁻¹) and specific growth rate (range: 1.26-1.87% day⁻¹) between fish fed with the control diet and those fed with diets containing 10% of AM. FCR increased from 1.44±0.03 to 2.35±0.14, with differences being significant at AM level exceeding 20% in diets ($P < 0.05$). Apparent net protein utilization decreased as AM increased, with significant differences between groups of diets containing more than 20% of AM ($P < 0.05$).

Body composition of fish and nutrient retention

No significant differences were found in body crude protein (range: 12.0-12.3%

fresh matter) and body P (range: 0.62-0.68% dry matter) content among fish fed with all the experimental diets ($P > 0.05$). Total P or N supplied with feed varied among dietary treatment (Table 3). TP and TN supplied decreased as AM increased in the diets, from 62.5 ± 2.0 to 33.4 ± 1.4 for TP and from 259.4 ± 8.5 to 203.7 ± 8.8 for TN ($P < 0.05$). The nutrient output as wastes decreased significantly with increasing AM in diets ($P < 0.05$). The evaluation as percentage of the amount supplied show no significant difference for TP ($P > 0.05$), whereas TN increased from 69.5% to 80.7% ($P < 0.05$) when dietary AM increased from 0% to 50%, with significant difference when AM was greater than 20%.

DISCUSSION

In the present study, the high survival rate indicates that experimental conditions are within the acceptable range for the production of Nile tilapia. Values obtained are comparable to those reported by Abdel-Tawwab and Ahmad (2009) in tanks while they are greater than the findings of Abou et al. (2007a, 2007b) in ponds. The growth performances namely the SGRs are better than the values reported by Garduño-Lugo and Olvera-Novoa (2008) when finding Nile tilapia with peanut (*Arachis hypogea* L.) leaf as a partial replacement of dietary FM. Our results indicate that AM could be used at upto 10% in diets without affecting fish growth. The decrease in growth performance when AM level was greater than 10% agreed with Fasakin et al. (2001) and Garduño-Lugo and Olvera-Novoa (2008) who reported a decline in growth rate when *Azolla Africana* L. and *Arachis hypogea* were used as FM partial replaces in *O. niloticus* diets. The main causative factor here could be the relative low digestibility of AM in *O. niloticus* and consequent lower nutrient availability in diets with high level of AM, as only 71.3% of protein in that fern is digestible (Micha and

Leonard, 2001-2), which is lower than the rate of about 90-92% generally reported for FM in this fish species (Sklan et al., 2004).

There are many dietary effects on crude protein and P levels in all experimental fish carcasses. These results agreed with findings reported in Nile tilapia by Garduño-Lugo and Olvera-Novoa (2008) in floating cages and Schneider et al. (2004) in aquaria. These last authors did not find significant differences in fish body P when feeding Nile tilapia in aquaria with plant-based diets (with 0.19-2% P content) as substitutes. According to Schneider et al. (2004), the digestibility of phosphorus from plant-based diets in *O. niloticus* is generally higher than that of FM-diets; and Leonard et al. (1998) have found values ranging from 71.0 to 89.3% for digestibility coefficient of phosphorus from *A. filiculoides* in *Oreochromis aureus*, a species close to *O. niloticus*. But because of the high gastric acidity of Nile tilapia (Moreau, 1988) and the physiological response of fish that tend to keep nutrient to the minimum requirement, higher excess soluble P could be generated from the control diet and of which high amounts could be rapidly eliminated. So, little difference may occur in terms of quantity between the low available P extracted from the diets A₀ and the relatively high available P from AM-diets (which contained lower dietary P); this could lead to assimilation of a similar quantity of P by all types of fish. Such findings have been reported in rainbow trout by Nakashima and Leggett (1980).

From the environmental point of view, TP waste expressed as the percentage of TP supplied show unexpectedly similar results. This contrasts with Jahan et al. (2003), and Kaushik et al. (2004) who found a reduction in P discharge when feeding fish with diets containing lower P, in which FM was replaced by corn gluten meal. As there is no data on P discharge as a consequence of feeding Nile tilapia with *Azolla*, investigations must be continued to precise the effect of

Table 2: Growth, feed performance and body composition of Nile tilapia fed in concrete tanks with *Azolla*-diets. Data are mean \pm S.D. of three replicates.

Diets	A ₀	A ₁₀	A ₂₀	A ₃₀	A ₄₀	A ₅₀	
Initial weight (g)	16.4 \pm 0.0	16.4 \pm 0.0	16.4 \pm 0.0	16.4 \pm 0.0	16.4 \pm 0.0	16.4 \pm 0.0	
Final weight (g)	88.3 \pm 2.0 ^a	84.8 \pm 3.5 ^a	78.4 \pm 1.8 ^b	68.2 \pm 2.1 ^c	60.2 \pm 1.3 ^d	51.0 \pm 2.4 ^e	
Growth rate (g day ⁻¹)	0.80 \pm 0.02 ^a	0.76 \pm 0.04 ^a	0.69 \pm 0.02 ^b	0.58 \pm 0.02 ^c	0.49 \pm 0.01 ^d	0.38 \pm 0.03 ^e	
Specific growth rate (% day ⁻¹)	1.87 \pm 0.02 ^a	1.82 \pm 0.05 ^a	1.74 \pm 0.03 ^b	1.58 \pm 0.03 ^c	1.44 \pm 0.02 ^d	1.26 \pm 0.05 ^e	
Survival (%)	91.1 \pm 1.9	89.4 \pm 2.6	91.7 \pm 4.4	90.6 \pm 4.2	91.7 \pm 1.7	93.3 \pm 3.3	
FCR	1.44 \pm 0.03 ^a	1.49 \pm 0.03 ^a	1.56 \pm 0.06 ^a	1.79 \pm 0.10 ^b	1.98 \pm 0.05 ^c	2.35 \pm 0.14 ^d	
Apparent net protein utilization (%)	36.1 \pm 3.1 ^a	35.6 \pm 5.2 ^a	34.0 \pm 4.0 ^a	31.9 \pm 0.9 ^a	25.8 \pm 1.8 ^b	26.0 \pm 3.6 ^b	
<i>Body composition (%)</i>							Initial
Crude protein (% fresh matter)	12.3 \pm 0.9	12.2 \pm 0.3	12.3 \pm 0.7	12.0 \pm 0.5	12.2 \pm 0.6	12.1 \pm 0.9	10.2 \pm 0.0
Phosphorus (% dry matter)	0.65 \pm 0.03	0.65 \pm 0.05	0.68 \pm 0.06	0.65 \pm 0.09	0.64 \pm 0.04	0.62 \pm 0.06	0.40 \pm 0.01

^{a,b,c,d,e}In each line, means with no letters or with the same letters as superscripts are not significantly different ($P > 0.05$).

Table 3: Phosphorus and nitrogen balances in Nile tilapia fed *Azolla*. Data are mean \pm S.D. of three replicates.

Diets	A ₀	A ₁₀	A ₂₀	A ₃₀	A ₄₀	A ₅₀
P supplied (g tank ⁻¹)	62.5 \pm 2.0 ^a	57.1 \pm 2.8 ^b	55.0 \pm 3.1 ^b	48.7 \pm 1.6 ^c	43.8 \pm 1.1 ^d	33.4 \pm 1.4 ^e
N supplied (g tank ⁻¹)	259.4 \pm 8.5 ^a	248.0 \pm 12.2 ^a	242.6 \pm 13.5 ^{ab}	226.6 \pm 7.5 ^{bc}	215.5 \pm 5.6 ^{cd}	203.7 \pm 8.8 ^d
P retention rate (%)	9.94 \pm 0.63	9.99 \pm 1.02	10.4 \pm 1.0	9.30 \pm 1.64	8.85 \pm 0.45	8.87 \pm 0.83
N retention rate (%)	30.5 \pm 2.8 ^a	29.4 \pm 1.2 ^a	28.3 \pm 2.4 ^a	24.4 \pm 0.4 ^b	22.6 \pm 1.8 ^b	19.3 \pm 1.1 ^c
P retained (g tank ⁻¹)	6.22 \pm 0.44 ^a	5.72 \pm 0.84 ^a	5.73 \pm 0.46 ^a	4.54 \pm 0.90 ^b	3.87 \pm 0.10 ^{bc}	2.97 \pm 0.40 ^c
N retained (g tank ⁻¹)	79.2 \pm 9.1 ^a	73.0 \pm 5.4 ^a	68.8 \pm 9.1 ^a	55.4 \pm 2.5 ^b	48.8 \pm 4.9 ^{bc}	39.3 \pm 3.7 ^c
Total P waste (g tank ⁻¹)	56.3 \pm 1.9 ^a	51.4 \pm 2.1 ^b	49.3 \pm 3.1 ^b	44.1 \pm 1.2 ^c	40.0 \pm 1.2 ^d	30.4 \pm 1.0 ^e
Total N waste (g tank ⁻¹)	180.2 \pm 5.5 ^a	174.9 \pm 8.1 ^{ab}	173.8 \pm 6.5 ^{ab}	171.2 \pm 5.2 ^b	166.7 \pm 2.5 ^b	164.4 \pm 5.8 ^b
Total P waste (% supplied)	90.1 \pm 0.6	90.0 \pm 1.0	89.6 \pm 1.0	90.7 \pm 1.6	91.2 \pm 0.4	91.1 \pm 0.8
Total N waste (% supplied)	69.5 \pm 2.8 ^a	70.6 \pm 1.2 ^a	71.7 \pm 2.4 ^a	75.6 \pm 0.4 ^b	77.4 \pm 1.8 ^{bc}	80.7 \pm 1.1 ^c

^{a,b,c,d,e}In each line, means with no letters or with the same letters as superscripts are not significantly different ($P > 0.05$).

Azolla in low-polluting fish-feed. In contrary, TN waste increased with increasing AM in the diets, as a consequence of the relative lower digestibility of protein from the fern and the high digestibility and assimilation of that providing from FM. This probably cause the reduction of fish growth as explained above, consistency with Jahan et al. (2003) who stated that growth reflects the FCR and a better FCR indicates a lower discharge of unassimilated nutrient, and with Ballestrazzi et al. (1994) and Bergheim et al. (1996) who reported that FCR and nutrients released into the water partially depend on fish size and the type of culture system.

Conclusion

Based on the findings from this study, AM could be used at upto 10% in diets without adverse effects on growth performance. In low-polluting feed field however, further investigations must be implemented to formulate AM-diets that sustain growth while reducing P wasted by fish. Positively however, the undigested nitrogen from high AM-feed (AM level superior to 20%) is wasted and provided to water, which will be quite good in tropical marshland ponds where nitrogen is already limiting.

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