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Partial replacement of fish meal with *Azolla* meal in diets for Nile tilapia (*Oreochromis niloticus* L.) affects growth and whole body fatty acid composition

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ABSTRACT

The replacement of fishmeal (FM) with *Azolla* meal (AM) in diets on growth and fatty acid in Nile tilapia, *Oreochromis niloticus* (initial mean weight: 16.4 g) was studied. Fish were fed with six isonitrogenous (29.2% CP) and isoenergetic (16.9 kJ.g⁻¹) diets containing 0%, 10%, 20%, 30%, 40% and 50% AM respectively, as partial FM substitute. Diet A₀ acted as a control. The final weight (51.0-88.3 g), the weight gain (210.8-438.3%) and the SGR (1.3-1.9%day⁻¹) decreased when AM level higher than 10% in diets ($P < 0.05$). The FCR (1.44 to 2.35) significantly increased when AM level exceeded 20% ($P < 0.05$). The protein efficiency ratio and the apparent net protein utilization decreased consequently. The fatty acids in the whole fish were significantly influenced by the diets. The replacement of FM by AM produced fish with lower (n-3) highly unsaturated fatty acids mainly docosahexaenoic acid, and higher arachidonic acid ($P < 0.05$). The n-3/n-6 fatty acid ratio ranged from 0.08 to 0.17, and was lower in fish fed A₄₀ and A₅₀ ($P < 0.05$). The findings suggest that high AM in diets affects Nile tilapia growth and the fern could be incorporated in diets up to 30% without any adverse effect on fish fatty acid composition. However, alternative additional source of lipid must be used to improve the quality of fish fed AM-diets.

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Keywords: *Azolla*, Fish meal replacement, Growth, Fatty acids, *Oreochromis niloticus*, bioconversion.

INTRODUCTION

As the global production of fish meal was stabilized at 6 to 7 million tones since 1985, the prices of that feed ingredient has

risen (FAO, 2006). Consequently, research on the use of non-conventional sources of protein as ingredients to replace FM in fish feeds has received increasing attention by fish

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nutritionists around the world. Several vegetable sources have shown to be valuable to replace FM in fish diets, either partially or in total substitution. The use of *Sesbania* seeds (*Sesbania aculeata* L.), *Moringa* leaf (*Moringa oleifera* Lamarck), *Mucuna* seeds (*Mucuna pruriens* L.), duckweed (*Spirodela polyrrhiza* L. Schleiden), *Azolla* Lamarck, etc. in diets for *O. niloticus* had yielded encouraging results (Fasakin et al., 2001; Hossain et al., 2002; Siddhuraju and Becker, 2003; Afuang et al., 2003; Fiogbé et al., 2004). Interests on the use of the aquatic fern *Azolla* in fish farming are recent. Indeed, due to its symbiotic relationship with the cyanobacteria named *Anabaena azolla* Strasburger, this small-leaf free-floating fern commonly found in aquatic ecosystems of tropic and sub-tropic regions, fix the atmospheric nitrogen and is naturally rich in protein, show a good amino acids profile and contained some vitamins and minerals (Leonard, 1997). Research on the use of *Azolla* as a feed ingredient in fish farming had been conducted in controlled conditions (Leonard, 1997; Micha and Leonard, 2001-2; Fiogbé et al., 2004), as well as in ponds (Abou et al., 2007). So far, *Azolla* research in that area is strongly focused on growth performance and digestibility in *O. niloticus* and *O. aureus* Steindachner (Leonard et al., 1998; Micha and Leonard, 2001-2). As a criterion for sustainable aquaculture, the nutritional quality of the fish produced should also be taken into account. Indeed, the market value of cultured fish largely depends on their quality and feed composition is one of the factors that control quality (Shearer, 2001). It is currently well established that increased fish consumption in human is associated with decreased mortality, as well as morbidity from cardiovascular disease and coronary heart disease (Arts et al., 2001). And the links between fish as food and human health are strongly related to the fatty acid composition of the food (Steffens, 1997). Especially, (n-3)

fatty acid series are known to improve neurological function in newborn infants (Maina et al., 2003). Therefore, the fatty acid profile of fish produced could be another tool to evaluate the relative suitability of feeding with *Azolla* and could serve as an indicator of nutritional quality, especially for the consumer's health. To date, there is no data on the effect of feeding with *Azolla* on fish fatty acid composition.

This study aimed at analysing the effects on growth and the changes in fatty acids of Nile tilapia fed with increasing amount of AM as alternative source of dietary protein in replacement of FM.

MATERIALS AND METHODS

Azolla culture

The strain of *Azolla* used is *A. filiculoides* Lamarck. The fern was produced in one pond of 100 m². For use, effort was made to always harvest the fern in its linear phase of growth, i.e. when the biomass of fresh matter is about 1-2 kg m⁻² (Van Hove, 1989), in order to entirely conserve its nutritional value. The linear phase of growth in the culture conditions is reached in about 10 days after inoculation (personal observation), depending however on the density of inoculation. Before its use in diets, the fern was harvested, washed with water to remove residual soil and debris, sun-dried (32-35 °C) and ground (200 µm). Then, the meal was stored in air-tight plastic containers and kept in a refrigerator (+ 4 °C) until used for diet production.

Fish, diets and feeding regimes

The experiment was carried out for 90 days in station (6°25'1.53"N 2°20'42.2"E) located at the University of Abomey-Calavi, Benin (West Africa). Sixty male fingerlings of Nile tilapia *Oreochromis niloticus* (initial mean weight = 16.4 g) from Lassissi fish farming center were stocked in each of eighteen 600 L square concrete tanks.

Fish were fed with six isonitrogenous (29.2% CP) and isoenergetic (16.9 kJ.g⁻¹) diets formulated to contain 0% (A₀), 10% (A₁₀), 20% (A₂₀), 30% (A₃₀), 40% (A₄₀) and 50% (A₅₀) of AM using locally available ingredients and the freshwater fern *A. filiculoides*. Formulation and proximate composition of experimental diets are given in Table 1. The experimental diets were prepared according to the procedures described in Abou et al. (2007), broken into smaller pellets (5 mm) and preserved in a refrigerator (+ 4 °C) until their distribution to fish.

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. The used water coming from the rearing tanks flowed respectively through a 300 L tank for decantation, a 300 L tank for biological filtration and a 3 m³ tank containing a pump (Riverdrain 7800) which was able to fill continuously the initial 1m³ tank installed at a 2-m height from the floor. The experimental tanks were randomly assigned to six groups, each in three replicates (6 x 3 tanks) was attributed one of the diets.

Fish were fed every day with the daily ration calculated based on the optimal daily ration rate estimation according to Melard (1986). Daily rations were divided into two parts, each hand-distributed at 8:00 h and 16:00 h respectively. The daily rations were adjusted fortnightly according to fish biomass in each tank.

Sampling and chemical analysis

Six fish per tank were randomly sampled and stored at -20 °C for analysis. Whole fish were mashed using a Robot coupe food processor and whole body crude lipid was determined according to Folch et al. (1957). The diets used were reduced in meal and preserved at -20 °C for biochemical analysis. They were analyzed for dry matter (AOAC, 1990), crude protein (Nitrogen x

6.25, Kjeldahl method), crude lipid (Folch et al., 1957) and crude ash content (incineration for 24 h at 550 °C in a muffle furnace).

Amino acid composition was determined by ion-exchange chromatography using a Waters HPLC (Waters, Milford, MA, USA), column AccQ-Tag 3.9 x 150, following hydrolysis of samples with 6 M HCl at 110 °C under an atmosphere of N₂ for 18 hrs. Fatty methyl esters were prepared after transesterification with anhydrous methanol containing 1% sulphuric acid and 0.01% (w/v) butylated hydroxytoluene (BHT) for 16 h at 50 °C (Christie, 1982). They were separated and quantified by gas chromatography (GC Trace, 2000, Thermo Quest, Carlo Erba, Italy), using a 30 m x 0.32 mm capillary column (FAME-WaxTM, USA).

Statistical analysis

Data of growth performance, body composition and fatty acids in fish were subjected to a one-way analysis of variance (ANOVA) to test the effect of replacement of FM by AM on growth and fatty acid composition of whole fish (n = 3). Differences between means were determined by Duncan's Multiple Range test (Duncan, 1955) and were considered to be significant when *P*-values were < 0.05. Before analyses, homogeneity of variance was checked using the "Hartley test" (Hartley, 1959) and all data were log-transformed. All analyses were done using the statistical package SPSS version 17.0 for windows (SPSS, Chicago, Illinois, USA).

RESULTS

Amino acid and fatty acid composition of *Azolla* and diets

Essential amino acid (EAA) of *A. filiculoides* and experimental diets are presented in Table 2. Compared to the requirements of Tilapia (NRC, 1993), *A. filiculoides* is poor in methionine. This amino acid is also deficient in diets A₃₀, A₄₀ and A₅₀. The levels of the other EAA in *Azolla* as in

different experimental diets meet the requirements for Tilapia (NRC, 1993).

The percentages of fatty acids in *A. filiculoides*, in the different feedstuffs and in experimental diets are presented in Tables 3 and 4. The most abundant fatty acids found in *A. filiculoides* were palmitic acid (16:0), oleic acid (18:1n-9) and linoleic acid (LA, 18:2n-6).

All experimental diets showed similar percentages for palmitic acid (27.3-29.3%), stearic acid 18:0 (10.1-12.4%) and oleic acid (16.4-20.4%). Percentages of 14:0 (2.2-6.9%), 16:1n-7 (2.0-4.4%), 18:3n-3 (1.0-2.4%), 20:5n-3 (0.6-1.5%), 22:5n-3 (0.1-0.6%) and 22:6n-3 (0.1-0.4%) decrease with decreasing FM in diets, whereas LA (18:2n-6) and arachidonic acid (ARA, 20:4n-6) levels tend to increase with increasing AM, with values ranging from 24.9% to 29.9% and from 1.4% to 4.1%, respectively.

Growth and body composition of experimental fish

As presented in Table 5, no significant differences were found in survival rate ($P > 0.05$). The growth rates and feed utilization efficiency of *O. niloticus* were significantly affected ($P < 0.05$) by the dietary AM level (Table 5). The final mean weight (range: 51.0-88.3 g) and the weight gain (range: 210.8-438.3%) decreased as AM level higher than 10% in the diets. The FCR (range: 1.44-2.35) increased when AM level is superior to 20% in the diets. Accordingly, PER and ANPU decreased when the level of AM was higher than 10% and 20%, respectively.

Data on Nile tilapia body composition are also presented in Table 5. No significant differences were found in moisture, crude protein and crude ash among fish fed with all experimental diets ($P > 0.05$). Carcass lipid decreased significantly when AM level exceeded 20% in diets ($P < 0.05$).

The concentrations in fatty acids of the whole body of the experimental fish are presented in Table 6. Data showed that fatty

acid composition in fish was influenced by the composition in the diet fed. Indeed, fatty acids found in high concentrations in the diets were also the most abundant in fish. 16:0, 18:1n-9 and 18:2n-6 were the most abundant fatty acids in the diets and fish (Tables 4 and 6), and levels of these fatty acids in fish ranged from $28.2 \pm 0.9\%$ to $30.5 \pm 2.3\%$ for 16:0, $15.4 \pm 2.8\%$ to $18.4 \pm 1.6\%$ for 18:1n-9 and $17.5 \pm 2.5\%$ to $22.6 \pm 2.7\%$ for 18:2n-6. Palmitoleic acid (16:1n-7) and vaccenic acid (18:1n-7) levels in fish decreased significantly ($P < 0.05$) as AM level increased in diets, with values decreasing from $4.4 \pm 0.5\%$ to $2.7 \pm 0.3\%$ for 16:1n-7 and from $3.6 \pm 0.3\%$ to $2.7 \pm 0.3\%$ for 18:1n-7.

LA level decreases significantly in fish fed with high AM in diets whereas ARA increased ($P < 0.05$). The poly-unsaturated (n-3) fatty acids (n-3 PUFAs) group is the family of fatty acids that were presented at lower levels in fish. Level of linolenic acid (LLA, 18:3n-3) decreases in fish, the difference being significant when fish consumed diets containing more than 30% AM. Eicosapentaenoic acid (EPA) and clupanodonic acid levels varied significantly in fish, whereas a decreasing trend was found for docosahexaenoic acid (DHA) level ($P < 0.05$), the lowest values being observed in fish fed A_{40} and A_{50} .

Total saturated fatty acids levels were similar in all experimental fish ($P > 0.05$). The total of fatty acids of (n-3) series ranged from 2.61 to 4.51, the lowest values being obtained in fish fed A_{40} and A_{50} ($P < 0.05$). In contrary, these fish showed significantly higher values for (n-6) fatty acids series ($P < 0.05$). As a consequence, the ratio n-3/n-6 calculated (range: 0.08-0.17) were lower in fish fed A_{40} and A_{50} ($P < 0.05$).

Table 1: Formulation and proximate composition of experimental diets used for feeding Nile tilapia in concrete tanks for 90 days.

Diets	A ₀	A ₁₀	A ₂₀	A ₃₀	A ₄₀	A ₅₀
Ingredients (g.100g⁻¹ diet)						
Fish meal	30	25	20	15	10	5
<i>Azolla</i> 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
Proximate composition[‡]						
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

*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 *Azolla filiculoides*, respectively.[†]Nitrogen-Free Extract, calculated as: 100-(%protein + %lipid + %ash + %crude fibre).[‡]Calculated by using conversion factors of 23.0, 38.1 and 17.2 kJ.g⁻¹ for protein, lipids and carbohydrates (Tacon, 1990).

DM : dry matter.

Table 2: Essential amino acid (EAA) composition (% on dry matter basis) of *A. filiculoides* and experimental diets used for feeding Nile tilapia in concrete tanks for 90 days.

Amino acids	<i>Azolla</i> [¶]	A ₀	A ₁₀	A ₂₀	A ₃₀	A ₄₀	A ₅₀	Requirement [†]
Cystine	0.22	0.49	0.46	0.42	0.44	0.41	0.40	0.15
Methionine	0.39	0.80	0.77	0.76	0.71	0.66	0.60	0.75
Valine	1.49	1.83	1.74	1.73	1.79	1.74	1.81	0.78
Isoleucine	1.24	1.42	1.35	1.36	1.43	1.39	1.46	0.87
Leucine	2.12	2.70	2.54	2.55	2.66	2.51	2.64	0.95
Tyrosine	0.87	0.85	0.76	0.78	0.91	0.96	0.97	0.50
Phenylalanine	1.68	1.89	1.72	1.82	1.86	2.05	2.07	1.05
Histidine	0.57	0.86	0.78	0.79	0.81	0.83	0.85	0.48
Lysine	1.31	1.69	1.63	1.58	1.56	1.51	1.46	1.43
Arginine	1.51	2.82	2.53	2.54	2.50	2.64	2.74	1.18
Threonine	1.34	1.36	1.24	1.25	1.33	1.39	1.48	1.05
Tryptophan	0.41	0.32	0.34	0.32	0.36	0.34	0.38	0.28

[†]Essential amino acid requirements (g.100 g⁻¹ feed) for tilapia taken from NRC (1993).[¶]*Azolla filiculoides* strain was used.

Table 3: Fatty acid composition (% total fatty acids) of *A. fliculoides* and ingredients used in the experimental diets.

Fatty acid	<i>Azolla</i> meal	Fish meal	Maize bran	Cotton cake	Brewery draff
14:0	5.1	14.2	0.4	1.0	0.7
16:0	45.7	35.7	10.8	24.7	22.9
18:0	2.2	6.5	3.6	2.9	3.6
16:1n-7	4.5	13.0	0.5	0.6	0.6
18:1n-9	16.1	11.1	33.6	18.0	16.0
18:1n-7	2.3	3.6	-	0.9	-
18:2n-6	18.2	3.7	50.4	45.8	48.9
18:3n-3	-	4.2	0.8	0.4	3.3
20:4n-6	5.9	3.2	-	4.8	4.0
20:5n-3	-	2.1	-	0.2	-
22:5n-3	-	1.5	-	0.2	-
22:6n-3	-	1.2	-	0.5	-

Table 4: Percentages of fatty acids (% of total fatty acids) in diets containing gradual level of *Azolla* fed to Nile tilapia reared for 90 days in concrete tanks.

Diet	A ₀	A ₁₀	A ₂₀	A ₃₀	A ₄₀	A ₅₀
SFA						
14:0	6.9	6.6	5.0	4.6	2.9	2.2
16:0	29.1	28.7	28.4	28.6	29.3	27.3
18:0	10.1	11.4	12.4	10.8	11.5	11.2
Total SFA	46.1	46.6	45.8	44.1	43.7	40.7
MUFA						
16:1n-7	4.4	4.1	3.7	2.9	2.5	2.0
18:1n-9	16.4	16.8	17.4	18.4	18.4	20.4
18:1n-7	1.9	1.7	1.3	1.8	1.8	1.0
Total MUFA	22.6	22.6	22.4	23.1	22.7	23.4
PUFA						
18:2n-6	24.9	24.3	24.8	26.1	27.0	29.9
18:3n-3	2.4	2.4	2.1	1.5	1.5	1.0
20:4n-6	1.4	1.8	3.1	3.5	3.9	4.1
20:5n-3	1.5	1.3	0.9	1.0	0.8	0.6
22:5n-3	0.6	0.6	0.6	0.3	0.1	0.2
22:6n-3	0.4	0.4	0.3	0.3	0.2	0.1
Total PUFA	31.2	30.7	31.7	32.8	33.6	35.9

SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 5: Growth, feed performance and body composition (% fresh matter basis) of Nile tilapia fed in concrete tanks with diets containing increasing level of *Azolla filiculoides* for 90 days. Data are mean \pm S.D. of three replicates. A₀ to A₅₀: diet with 0% to 50% *Azolla*.

Diets	A ₀	A ₁₀	A ₂₀	A ₃₀	A ₄₀	A ₅₀
Initial mean 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 mean 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
Weight gain (%)	438.3 \pm 12.0 ^a	416.8 \pm 21.4 ^a	378.2 \pm 11.1 ^b	316.0 \pm 12.7 ^c	267.2 \pm 7.8 ^d	210.8 \pm 14.7 ^e
SGR (% 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 rate (%)	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 ^d	1.49 \pm 0.03 ^d	1.56 \pm 0.06 ^d	1.79 \pm 0.10 ^c	1.98 \pm 0.05 ^b	2.35 \pm 0.14 ^a
Protein efficiency ratio	2.42 \pm 0.06 ^a	2.37 \pm 0.04 ^{ab}	2.25 \pm 0.05 ^b	1.99 \pm 0.08 ^c	1.79 \pm 0.03 ^d	1.52 \pm 0.09 ^e
Apparent net protein utilization	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
Body composition (% fresh matter)						
Dry matter (%)	22.4 \pm 0.6	22.2 \pm 0.5	22.3 \pm 1.0	22.2 \pm 0.4	22.1 \pm 1.0	21.5 \pm 1.6
Crude protein (%)	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
Crude lipid (%)	5.31 \pm 0.01 ^a	5.27 \pm 0.11 ^a	5.14 \pm 0.67 ^{ab}	4.72 \pm 0.15 ^{bc}	4.54 \pm 0.12 ^c	4.43 \pm 0.05 ^c
Crude ash (%)	4.80 \pm 0.81	4.73 \pm 0.22	4.80 \pm 0.82	5.46 \pm 0.17	5.28 \pm 0.30	4.80 \pm 1.38

^{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 6: Means \pm S.D. (% of total fatty acid) of fatty acid levels in the whole body of Nile tilapia *O. niloticus* fed in tanks with diets containing gradual level of *Azolla filiculoides*. A₀ to A₅₀: diet with 0% to 50% *Azolla*.

Diets	A ₀	A ₁₀	A ₂₀	A ₃₀	A ₄₀	A ₅₀
<i>Fatty acid</i> *						
SFA						
14:0	4.14 \pm 0.42 ^a	3.85 \pm 0.09 ^a	3.59 \pm 0.67 ^a	3.62 \pm 1.00 ^a	3.48 \pm 0.13 ^a	2.53 \pm 0.20 ^b
16:0	28.2 \pm 0.9	28.8 \pm 0.4	29.2 \pm 0.9	29.8 \pm 1.4	29.8 \pm 0.4	30.5 \pm 2.3
18:0	9.71 \pm 1.58	10.7 \pm 0.7	9.59 \pm 1.81	11.2 \pm 3.9	9.94 \pm 0.37	11.9 \pm 1.2
Total SFA	42.1 \pm 1.9	43.4 \pm 1.2	42.4 \pm 1.9	44.6 \pm 4.5	43.2 \pm 0.6	44.9 \pm 3.7
MUFA						
16:1n-7	4.43 \pm 0.53 ^a	4.17 \pm 0.06 ^{ab}	4.53 \pm 0.42 ^a	3.56 \pm 0.72 ^{bc}	3.15 \pm 0.08 ^{cd}	2.69 \pm 0.29 ^d
18:1n-9	18.4 \pm 1.6	17.5 \pm 1.7	18.2 \pm 1.3	17.4 \pm 2.9	18.2 \pm 0.6	15.4 \pm 2.8
18:1n-7	3.61 \pm 0.35 ^a	3.21 \pm 0.02 ^{ab}	2.69 \pm 0.59 ^b	3.07 \pm 0.12 ^{ab}	2.48 \pm 0.57 ^b	2.71 \pm 0.34 ^b
Total MUFA	26.5 \pm 1.9 ^a	24.9 \pm 1.8 ^{ab}	25.4 \pm 1.2 ^a	24.1 \pm 3.8 ^{ab}	23.8 \pm 0.9 ^{ab}	20.8 \pm 3.1 ^b
PUFA						
18:2n-6 (LA)	22.6 \pm 2.7 ^a	22.2 \pm 0.9 ^{ab}	23.5 \pm 2.5 ^a	21.3 \pm 4.0 ^{abc}	17.8 \pm 0.2 ^{bc}	17.5 \pm 2.5 ^c
18:3n-3 (LLA)	1.55 \pm 0.51 ^a	1.23 \pm 0.28 ^{ab}	1.11 \pm 0.20 ^{ab}	0.95 \pm 0.07 ^b	0.81 \pm 0.13 ^b	0.25 \pm 0.04 ^c
20:4n-6 (ARA)	4.32 \pm 1.45 ^a	5.28 \pm 1.01 ^a	4.45 \pm 2.00 ^a	6.25 \pm 2.95 ^a	12.1 \pm 0.4 ^b	14.2 \pm 0.9 ^b
20:5n-3 (EPA)	0.53 \pm 0.05 ^d	0.91 \pm 0.09 ^{ab}	1.18 \pm 0.30 ^a	0.79 \pm 0.20 ^{bc}	0.71 \pm 0.03 ^{bcd}	0.56 \pm 0.13 ^{cd}
22:5n-3	1.24 \pm 0.21 ^a	0.92 \pm 0.08 ^{ab}	1.13 \pm 0.31 ^{ab}	0.96 \pm 0.20 ^{ab}	0.77 \pm 0.16 ^b	1.08 \pm 0.12 ^{ab}
22:6n-3 (DHA)	1.22 \pm 0.39 ^a	1.21 \pm 0.07 ^a	0.91 \pm 0.30 ^{ab}	1.03 \pm 0.03 ^{ab}	0.72 \pm 0.05 ^b	0.72 \pm 0.05 ^b
Total PUFA	31.4 \pm 0.5 ^b	32.8 \pm 0.8 ^b	32.3 \pm 1.0 ^b	31.3 \pm 0.8 ^b	32.9 \pm 0.3 ^{ab}	34.3 \pm 1.7 ^a
\sum n-3 PUFA	4.53 \pm 1.11 ^a	4.27 \pm 0.40 ^a	4.33 \pm 0.48 ^a	3.73 \pm 0.29 ^{ab}	3.02 \pm 0.19 ^{bc}	2.61 \pm 0.20 ^c
\sum n-6 PUFA	26.9 \pm 1.3 ^c	27.5 \pm 0.8 ^c	27.9 \pm 0.7 ^{bc}	27.6 \pm 1.0 ^c	29.9 \pm 0.5 ^{ab}	31.7 \pm 1.9 ^a
\sum n-3 / \sum n-6	0.17 \pm 0.05 ^a	0.16 \pm 0.02 ^a	0.15 \pm 0.02 ^a	0.14 \pm 0.02 ^a	0.10 \pm 0.01 ^b	0.08 \pm 0.01 ^b

*Values are means of three replicates

SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid; LA, linoleic acid; LLA, linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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

DISCUSSION

The present study showed that the level of AM in diets affects the growth and feed utilization efficiency of Nile tilapia. High level of AM in diets resulted in lower growth. These trends of observed growth variation and FCR followed the results reported by Fasakin et al. (2001) and Fiogbé et al. (2004). The results were also comparable to those reported in several studies with the same purpose of replacing FM by plant protein in Nile tilapia feeding (Hossain et al., 2002; Olvera-Novoa et al., 2002). According to Cole and Van Lunen (1994), inadequate levels of indispensable amino acids could result in depression of food intake and growth. Deficiencies of one or more amino acids are known to limit the synthesis of protein, growth, or both (Cole and Van Lunen, 1994). The limiting EAA in *A. filiculoides* is methionine, with a requirement for optimal growth in tilapia set at 0.75% (NRC, 1993). In the present study, increasing AM level in diets resulted obviously in lower dietary methionine supply. However, due to sparing action of cystine whose values in experimental diets were higher than that the required in tilapia diets, the lower methionine could not be a major reason for lowered growth in fish fed diets A₂₀ to A₅₀.

The proof was given by the observed lowered growth with fish A₂₀ that was fed with sufficient methionine in their diet. Therefore, the main explanation to the lower growth performance in high AM-fed fish could be the lower nutrient digestibility in the fish. Indeed, Micha and Leonard (2001-2) found that only 64.6% of dry matter of *A. filiculoides* is digestible in *O. niloticus*, in which only 71.3% of protein is digestible, lower than the rate of about 90-92% generally reported for FM in that species (Watanabe et al., 1996; Sklan et al., 2004). As the fern increased in the diets, the nutrients become progressively unavailable to experimental fish and could lead to a lower growth performance.

In the present study, no negative effects were found in moisture and crude protein of all experimental fish carcasses. Similar findings had been reported in Nile tilapia by Schneider et al. (2004). Conversely, lipid deposition in fish decreased significantly

in fish fed diets containing more than 20% of AM. These results were in agreement with those reported by Hossain et al. (2002) and Siddhuraju and Becker (2003) who successfully used *Sesbania* and *Mucuna* seed meal, respectively, in diets for *O. niloticus*. The possible explanations for these variations could be the reduction of FM in diet and the lower contribution by plant protein that contained a high amount of non-digestible cuticles (Afuang et al., 2003).

The percentages of myristic acid, stearic acid, palmitoleic acid, vaccenic acid, LLA, EPA, clupanodonic acid and DHA in diets decreased with decreasing FM, certainly because of the high levels of these fatty acids in FM, compared to AM. The increasing trends of LA and ARA levels in experimental diets are also probably due to the increase of AM, which reaches a higher level of that fatty acid compared to FM (Table 3). As far as we know, this is the first reported study on the effects of replacement of FM by AM on fatty acid composition of Nile tilapia, a tropical omnivorous fish. Data showed that the relative composition of fatty acids in the whole body of fish was in accordance with the profiles of the main dietary items. Similar results had been reported in many studies in which sunflower cake and meat meal was used as FM substitutes (Maina et al., 2003; Williams et al., 2003). These results have also been confirmed in studies linking dietary lipid from several sources to fatty acid profile of fish, mainly Atlantic salmon *Salmo salar* L. (Bell et al., 2002), Rainbow trout *Onchorhynchus mykiss* Walbaum (Caballero et al., 2002) and Murray cod *Maccullochella peelii peelii* (Francis et al., 2006). Similar findings were also obtained with *O. niloticus* fed with sunflower cake and corn oil in replacement of FM (Maina et al., 2003) and with red hybrid Tilapia *Oreochromis* sp. receiving palm oil-based diets (Bahurmiz and Ng, 2007). The decrease observed in the levels of 14:0 and 16:1n-7 fatty acids in fish were probably due to the decrease of the level of FM in diets, which has a higher content of both fatty acids than are present in AM. Total saturated fatty acids were deposited in fish at a relatively uniform concentration of 42.1 ± 1.9 to $44.9 \pm 2.7\%$, confirming the observations of Bell et al. (2002) and Bahurmiz and Ng (2007) that

fish might maintain a constant level of saturated fatty acids.

In contrast, the level of ARA, clupanodonic acid and DHA in fish are higher than those determined in experimental diets. According to several reports, fatty acid profiles in cultured fish are influenced by the dietary contribution and the ability of experimental fish to *de novo* synthesize other fatty acids. In a particular case, it was shown that tilapia species possess enzymes that desaturate and elongate the dietary fatty acids of the (n-6) and (n-3) series after they are absorbed. The high values for ARA in fish compared to their levels in diets may be the result of the bioconversion of LA, as Maina et al. (2003) also found in *O. niloticus*. However, data obtained here showed that this conversion is more pronounced in fish fed with high AM in their diets. This situation is either because of the high content of LA in AM-based-diets or probably due to antagonistic effects in high (n-3) fatty acid diets (FM-based). Indeed, Kucska et al. (2006) stated that because of the common enzymes in the metabolic pathways of both (n-3) and (n-6) series of fatty acids, the (n-3) fatty acids like LLA, EPA and DHA may inhibit the desaturation and elongation process of LA in fish fed with FM-based-diets. On the other hand, it is well established that many freshwater fish (Anderson and Arthington, 1992), salmonids (Dosanjh et al., 1998) and rainbow trout (Mourente and Tocher, 1998) can readily convert LLA to EPA and DHA using metabolic pathways similar to those occurring in higher vertebrates. According to Maina et al. (2003) quoting Olsen et al. (1990) and Takeuchi et al. (1991), Tilapia possesses desaturation and elongation enzymes that efficiently convert C18 PUFAs to longer chain PUFAs. It is plausible and our findings suggest this fact, that LLA was converted to clupanodonic acid and DHA. Despite this bioconversion, the levels of the latter fatty acids decreased in fish when increasing the amounts of AM fed, closely reflecting the tendency in the distributed diets. Consequently, the sum of (n-3) fatty acid levels is significantly lower in fish fed diets A₄₀ and A₅₀ while the sum of (n-6) fatty acid levels was higher in those fish. As result, the values of n-3/n-6 ratios is lower in fish fed A₄₀

and A₅₀, suggesting similar nutritional quality of the fish until 30% of AM in diets.

Conclusion

From this study, high level of AM in diets for Nile tilapia affects growth performances and feed utilization due to lower nutrient digestibility in the fish. In fish fed diets containing AM in gradual replacement of FM, significantly higher deposition of ARA and lower (n-3) fatty acids in fish was resulted. Levels of these fatty acids in fish were influenced by their levels in diets, and the ability of Nile tilapia to bio-convert C18 PUFAs to HUFAs. The findings suggest the feasibility of the replacement of FM with AM up to 30% in the diet, without affecting significantly the nutritional quality of Nile Tilapia. However, feeding that fish in ponds or reaching alternative additional sources of lipids that is rich in n-3 fatty acids could be useful to improve growth and nutritional quality of Nile tilapia fed *Azolla*-based-diets.

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