

# Approximate Compositional Values and Tissue Fatty Acid Profiles of Nile Tilapia (*Oreochromis niloticus* L.) Fed *Azolla*-Diets in Earthen Ponds\*

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## ABSTRACT

The approximate general composition and the fatty acid profile of Nile tilapia fed *Azolla*-diets in ponds were studied for 90 days. Six isonitrogenous (29.2% CP) and isoenergetic (16.9 kJ·g<sup>-1</sup>) diets were formulated to contain 0% (A<sub>0</sub>), 10% (A<sub>10</sub>), 20% (A<sub>20</sub>), 30% (A<sub>30</sub>), 40% (A<sub>40</sub>) and 50% (A<sub>50</sub>) of *Azolla* meal (AM), as partial fish meal (FM) substitutes. Diet A<sub>0</sub> without AM served as a control. Fish growth decreased as AM level exceeded 20% in diets ( $P < 0.05$ ). Dry matter and crude protein showed no significant differences ( $P > 0.05$ ). Crude lipid was significantly lower in fish fed A<sub>50</sub> and significant differences were also found in crude ash ( $P < 0.05$ ). Linolenic acid (LLA) decreased significantly when AM level in diets increased ( $P < 0.05$ ). In contrast, arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) showed significantly higher values in fish fed high AM ( $P < 0.05$ ). The  $n - 3/n - 6$  ratio ranged from 0.35 to 0.49, with values being significantly higher in fish fed A<sub>20</sub>, A<sub>30</sub> and A<sub>50</sub>. High level of the fern reduces growth without negatively affecting fatty acid in fish. Fish PUFA, especially the ( $n - 3$ ) fatty acids, are affected positively, even when fed 50% AM, which is good for the quality of the fish produced in regard to the benefits for the health of consumers.

**Keywords:** *Azolla Filiculoides*, Fish Meal Replacement, *Oreochromis Niloticus*, Fatty Acid, Natural Food, Earthen Pond

## 1. Introduction

Aquaculture productions continue to increase, as a challenge to meet the ever-increasing demand for fish products worldwide. However, aquaculture enterprises are faced with the need to resolve the problem of increasing demand for fish meal (FM). Indeed, according to IFFO [1], global production of FM has stabilized at 6 to 7 million tonnes since 1985. Consequently, the prices has rising, driving research in the feed industry towards finding substitutes [2]. *Azolla*, a small floating freshwater fern that has been successfully used recently in fish farming [3-7] appears a good candidate. So far, research on *Azolla* in fish farming has strongly focused on its effects on growth performance and digestibility. No research on the nutritional quality of the fish produced is available, especially regarding fatty acid composition.

Yet, there are concerns that high replacement of FM could adversely affect the marketability of the fish, because of consumer perception of altered taste or reduced health benefits due to lowered ( $n - 3$ ) fatty acids content [8,9]. Indeed, the consumption of fish containing an adequate level of highly unsaturated ( $n - 3$ ) fatty acids ( $n - 3$  HUFAs) is favourable for human health [10,11], as these fatty acids series is believed to reduce the occurrence of heart diseases, strokes and various inflammatory injuries as well as to improve neurological function in newborn infants [12,13]. Many studies have been carried out to evaluate the effects of non-conventional ingredients used in diets as FM substitutes on fish fatty acid composition [13-15]. Findings have confirmed that body fatty acid composition to a large extent mirrors the dietary fatty acids composition [13,15]. However, biochemical observations show that live organisms, especially copepods and rotifers have a more suitable fatty

\*Tissue fatty acid of Nile tilapia fed *Azolla*.

acid pattern for aquaculture purposes, due to their high content in EPA and DHA [16,17], which must not be negligible in tilapia pond aquaculture.

As part of investigations examining the suitability of AM to replace FM in diets for Nile tilapia *O. niloticus*, the main objective of this study was to analyse proximate composition and whole body fatty acids profile in response to feeding this fish with gradual levels of *Azolla* in stagnant earthen ponds.

## 2. Material and Methods

### 2.1. Ponds, Fish and Feeding Regimes

The experiment was carried out for 90 days in a rural site (6°29'15.12"N 2°37'6.42"E, 13 m above the mean sea level) at Louho, a village located in Porto-Novo suburb, Benin (West Africa). In that site, eighteen small ponds of 30 m<sup>2</sup> (10 m × 3 m × 1 m, each) were constructed for the experiment. They were filled naturally from the water table. Fingerlings Nile tilapia of homogeneous size and age (initial body weight: 16.3 ± 0.1 g) were obtained

from Lassissi fish farming center in Porto-Novo, Benin. They were stocked at a density of 2 fish m<sup>-2</sup> (60 fish pond<sup>-1</sup>) to limit the influence of natural food in pond. The ponds were randomly assigned to 6 triplicate (6 × 3) groups, each set attributed one of the experimental diets. Diets were isonitrogenous (29.2% crude protein) and isoenergetic (16.9 kJ·g<sup>-1</sup>), formulated using locally available ingredients and the freshwater fern *Azolla filiculoides* Lamarck. They were formulated to contain 0% (A<sub>0</sub>), 10% (A<sub>10</sub>), 20% (A<sub>20</sub>), 30% (A<sub>30</sub>), 40% (A<sub>40</sub>) and 50% (A<sub>50</sub>) of AM. The *Azolla*-diets were compared with a control diet (A<sub>0</sub>) without AM. Formulation and proximate composition of experimental diets are given in **Table 1**. Experimental diets were prepared according to the procedure described in Abou *et al.* [6]. After sun-drying at 32°C - 35°C, the diet was broken into small particles (mm) and preserved in refrigerator (+4°C) until used for feeding fish.

Fish were fed every day according to Melard [18]. Daily rations were divided into two parts, each hand-

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

Diets	A <sub>0</sub>	A <sub>10</sub>	A <sub>20</sub>	A <sub>30</sub>	A <sub>40</sub>	A <sub>50</sub>
<i>Ingredients (g/100 g diet)</i>						
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
Cassava starch*	1	1	1	1	1	1
Salt (NaCl)	1	1	1	1	1	1
<i>Proximate 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 <sup>§</sup> (% DM)	9.9	10.0	10.2	10.4	10.5	10.8
NFE <sup>†</sup> (% DM)	35.7	36.8	38.7	39.4	41.0	42.2
Gross energy <sup>‡</sup> (kJ/g)	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

\*Binder; <sup>§</sup>Calculated according to Ovograin Feeds Depot, Abomey-Calavi, Bénin and Leonard [19] for ingredients and *Azolla filiculoides*, respectively; <sup>†</sup>Nitrogen-Free Extract, calculated as: 100 - (%protein + %lipid + %ash + %crude fibre); <sup>‡</sup>According to Tacon [20]; DM: dry matter.

distributed at 8:00 h and 16:00 h, respectively. Once every fortnight, fish in stock were sampled with a handling square-net, without entering the pond, and weighed. The daily ration was adjusted according to the actual body weight, and used for the next fortnight.

## 2.2. Biochemical Analysis

At the beginning of the experiment, thirty fish from the initial batch and six fish per pond at the end were randomly taken and mashed using a Robot coupe food processor. Subsamples were taken and stored at  $-20^{\circ}\text{C}$  for carcass analysis. Whole fish were mashed and whole body crude lipid was determined according to Folch *et al.* [21].

The diets used were reduced in meal and preserved at  $-20^{\circ}\text{C}$  for biochemical analysis. They were analyzed for dry matter [22], crude protein (Nitrogen  $\times$  6.25, Kjeldahl method), crude lipid [21] and crude ash content (incineration for 24 h at  $550^{\circ}\text{C}$  in a muffle furnace).

Amino acid composition was determined by ionexchange chromatography using a Waters HPLC (Waters, Milford, MA, USA), column AccQ-Tag 3.9  $\times$  150, following hydrolysis of samples with 6 M HCl at  $110^{\circ}\text{C}$  under an atmosphere of  $\text{N}_2$  for 18 h. 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  $50^{\circ}\text{C}$  [23].

They were separated and quantified by gas chromatography (GC Trace, 2000, Thermo Quest, Carlo Erba, Italy), using a 30 m  $\times$  0.32 mm capillary column (FAME-Wax<sup>TM</sup>, USA).

## 2.3. Statistical Analysis

Means for growth, proximate composition, and fish body fatty acids were analyzed using one-way Anova, after verifying the homogeneity of their variance [24]. Values for percentage data and ratios were log-transformed prior to analyses. When the effect was significant, comparisons between treatment means was run using Duncan's multiple range test [25] at  $P = 0.05$ . All analysis were done using SPSS program version 17.0 (SPSS, Chicago, Illinois, USA).

## 3. Results

### 3.1. Amino Acid of *Azolla* and Diets, and Fatty Acid Composition of Ingredients and Diets

Essential amino acids (EAA) of *Azolla filiculoides* and experimental diets are presented in **Table 2**. Compared to the requirements of tilapias [26], *A. filiculoides* is poor in methionine. This amino acid is also deficient in diets A<sub>30</sub>, A<sub>40</sub> and A<sub>50</sub>. The levels of the other EAA in *Azolla* as in different experimental diets meet the requirements for tilapia [26]. 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 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 (2.22% - 6.92%), 16:1n - 7 (1.95% - 4.42%), 18:3n - 3 (0.96% - 2.40%), 20:5n - 3 (0.61% - 1.52%), 22:5n - 3

**Table 2. Essential amino acid (EAA) composition (g/100 g feed) of *Azolla filiculoides* and experimental diets.**

Amino acids	<i>Azolla</i> <sup>‡</sup>	A <sub>0</sub>	A <sub>10</sub>	A <sub>20</sub>	A <sub>30</sub>	A <sub>40</sub>	A <sub>50</sub>	Requirement <sup>†</sup>
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

<sup>†</sup>Essential amino acid requirements (g/100 g feed) for tilapia taken from [26]; <sup>‡</sup>*Azolla filiculoides* strain.

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

Fatty acid	<i>Azolla</i> meal	Fish meal	Maize bran	Cotton cake	Brewery draff
14:0	5.05	14.2	0.40	0.98	0.70
16:0	45.7	35.7	10.8	24.1	22.7
18:0	2.24	6.49	3.55	2.80	3.63
16:1n-7	4.48	13.0	0.46	0.60	0.56
18:1n-9	16.1	11.1	33.6	17.5	15.8
18:1n-7	2.30	3.63	-	0.89	-
18:2n-6	18.2	3.72	50.4	52.8	53.2
18:3n-3	-	4.16	0.77	0.40	3.33
20:4n-6	5.93	3.16	-	-	-
20:5n-3	-	2.14	-	-	-
22:5n-3	-	1.46	-	-	-
22:6n-3	-	1.23	-	-	-

**Table 4. Percentages of fatty acids (% of total fatty acids) in diets containing gradual level of *Azolla* fed to Nile tilapia reared in ponds.**

Diet	A <sub>0</sub>	A <sub>10</sub>	A <sub>20</sub>	A <sub>30</sub>	A <sub>40</sub>	A <sub>50</sub>
<b>SFA</b>						
14:0	6.92	6.56	4.98	4.63	2.94	2.22
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
<b>MUFA</b>						
16:1n-7	4.42	4.11	3.69	2.87	2.47	1.95
18:1n-9	16.4	16.8	17.4	18.4	18.4	20.4
18:1n-7	1.87	1.72	1.33	1.80	1.83	1.04
Total MUFA	22.6	22.6	22.4	23.1	22.7	23.4
<b>PUFA</b>						
18:2n-6	24.9	24.3	24.8	26.1	27.0	29.9
18:3n-3	2.40	2.38	2.07	1.52	1.46	0.96
20:4n-6	1.39	1.75	3.15	3.49	3.92	4.15
20:5n-3	1.52	1.28	0.91	1.00	0.79	0.61
22:5n-3	0.61	0.58	0.56	0.35	0.15	0.17
22:6n-3	0.45	0.40	0.27	0.31	0.20	0.11
Total PUFA	31.2	30.7	31.7	32.8	33.6	35.9

(0.15% - 0.61%) and 22:6n - 3 (0.11% - 0.45%) decrease with decreasing FM in diets, whereas LA (18:2n - 6) and ARA (20:4n - 6) levels tend to increase with increasing AM.

### 3.2. Body Composition and Fatty Acid in Experimental Fish

Data on fish body composition at the beginning and at the end of the experiment are presented in **Table 5**. Moisture and crude protein were not significantly different among all the experimental diets ( $P > 0.05$ ). Carcass lipid content was significantly lower at high AM level (A<sub>50</sub>). Significant variations were found in crude ash ( $P < 0.05$ ).

Fatty acids in the whole body of experimental fish are presented in **Table 6**. In general, fatty acids found in high concentration 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 in fish. Levels of these fatty acids in fish ranged from 26.9% ± 1.1% to 29.1% ± 0.1% for 16:0, 15.0% ± 1.3% to 18.9% ± 0.9% for 18:1n - 9 and 18.2% ± 0.7% to 20.9% ± 1.2% for 18:2n - 6. Myristic acid (14:0) level decreased significantly as AM proportion increased in diets ( $P < 0.05$ ). The same trend of variation was found for palmitoleic acid (16:1n - 7) and vaccenic acid (18:1n - 7), with values decreasing from 4.49% ± 0.22% to 1.99% ± 0.42% for 16:1n - 7 and from 3.88% ± 0.12% to 2.62% ± 0.73% for 18:1n - 7.

Fish fed A<sub>0</sub> to A<sub>20</sub> are rich in 14:0, 16:1n - 7, 18:1n - 7, 18:1n - 9 and 18:3n - 3 fatty acids ( $P < 0.05$ ). Oppo-

site of this group, fish fed A<sub>30</sub> to A<sub>50</sub> showed higher percentages in 18:0, arachidonic acid (ARA, 20:4n - 6), eicosapentaenoic acid (EPA, 20:5n - 3) and docosahexaenoic acid (DHA, 22:6n - 3). EPA and DHA levels in those last fish showed a markedly higher concentration compared to their level in the diets provided. This leads to a significantly higher concentration of total PUFAs in fish fed AM-diets, mainly in (n - 3) and (n - 6) fatty acids. As a consequence, significant differences were found in the resulting n - 3/n - 6 ratios.

Identical values were obtained in fish fed A<sub>20</sub>, A<sub>30</sub> and A<sub>50</sub>, which were higher to the values recorded in fish fed A<sub>0</sub>, A<sub>10</sub> and A<sub>40</sub> ( $P < 0.05$ ).

### 4. Discussion

The results of the present study did not demonstrate any significant difference among fish fed AM-free diet (A<sub>0</sub>) and those fed either 10% (A<sub>10</sub>) or 20% (A<sub>20</sub>) of AM in their diets, in terms of final mean weight and SGR. These trends in growth variation were similar to those reported with other terrestrial and aquatic plants, as substitutes for FM. As in this study, El-Sayed [27], Fasakin *et al.* [28] and Fasakin *et al.* [4] reported reduced growth of Nile tilapia when *Azolla pinnata* R. Brown., Duckweed (*Spirodela polyrrhiza* L.) and *Azolla africana* Desv. were used, respectively. According to several studies, factors that limit the use of macrophytes in fish diets are their content of antinutrient factors, or a deficiency in amino-acids and phosphorus. Dato-Cajegas [29] pointed out phosphorus as the major mineral that limits Nile tilapia

**Table 5. Growth, feed performance and body composition (% fresh matter basis) of Nile tilapia fed in ponds with diets containing increasing level of *Azolla filiculoides* for 90 days. Data are mean ± S.D. of three replicates. A<sub>0</sub> to A<sub>50</sub>: diet with 0% to 50% *Azolla*.**

Amino acids	A <sub>0</sub>	A <sub>10</sub>	A <sub>20</sub>	A <sub>30</sub>	A <sub>40</sub>	A <sub>50</sub>	
Initial weight (g)	16.4 ± 0.2	16.3 ± 0.1	16.3 ± 0.2	16.2 ± 0.1	16.4 ± 0.1	16.4 ± 0.2	
Final weight (g)	135.9 ± 5.7 <sup>a</sup>	131.2 ± 6.3 <sup>a</sup>	126.4 ± 6.5 <sup>a</sup>	116.9 ± 4.1 <sup>b</sup>	95.3 ± 1.2 <sup>c</sup>	88.8 ± 5.7 <sup>c</sup>	
SGR (% day)	2.35 ± 0.04 <sup>a</sup>	2.31 ± 0.06 <sup>a</sup>	2.27 ± 0.06 <sup>ab</sup>	2.19 ± 0.04 <sup>b</sup>	1.96 ± 0.01 <sup>c</sup>	1.87 ± 0.06 <sup>c</sup>	
Survival (%)	86.7 ± 6.7	87.8 ± 4.2	90.0 ± 2.9	88.3 ± 2.9	85.0 ± 4.4	89.4 ± 4.2	
FCR	1.26 ± 0.11 <sup>b</sup>	1.26 ± 0.10 <sup>b</sup>	1.28 ± 0.08 <sup>b</sup>	1.35 ± 0.01 <sup>b</sup>	1.67 ± 0.12 <sup>a</sup>	1.68 ± 0.15 <sup>a</sup>	
<i>Whole body composition</i>							Initial
Dry matter (%)	23.0 ± 0.7	22.5 ± 0.8	23.1 ± 1.4	22.0 ± 0.9	22.3 ± 1.0	22.1 ± 1.1	21.0 ± 0.1
Crude protein (%)	12.9 ± 0.7	12.6 ± 0.8	12.3 ± 0.5	12.2 ± 0.2	12.1 ± 0.1	12.1 ± 0.7	10.2 ± 0.0
Crude lipid (%)	5.98 ± 0.23 <sup>a</sup>	5.67 ± 0.25 <sup>a</sup>	5.80 ± 0.56 <sup>a</sup>	5.75 ± 0.32 <sup>a</sup>	5.44 ± 0.02 <sup>ab</sup>	5.01 ± 0.19 <sup>b</sup>	4.40 ± 0.01
Crude ash (%)	4.09 ± 0.10 <sup>b</sup>	4.12 ± 0.19 <sup>b</sup>	4.88 ± 0.38 <sup>a</sup>	4.01 ± 0.16 <sup>b</sup>	4.34 ± 0.43 <sup>ab</sup>	5.01 ± 0.59 <sup>a</sup>	6.44 ± 0.47

SGR (%/day) =  $(\ln W_f - \ln W_i) \times 100 / \text{time (days)}$  where  $W_i$  and  $W_f$  are the initial and final mean wet weight in g. FCR = amount of feed supplied (g)/fresh weight gain of fish (g); FM, fresh matter. <sup>a,b,c</sup>In each line, means with no letters or with the same letters as superscripts are not significantly different ( $P > 0.05$ ).

**Table 6. Means ± S.D. of fatty acid levels (% of total fatty acids) in the whole body of Nile tilapia *O. niloticus* fed in ponds with diets containing gradual level of *Azolla filiculoides*. A<sub>0</sub> to A<sub>50</sub>: diet with 0% to 50% *Azolla*.**

Diets	A <sub>0</sub>	A <sub>10</sub>	A <sub>20</sub>	A <sub>30</sub>	A <sub>40</sub>	A <sub>50</sub>
<i>Fatty acid</i> *						
<b>SFA</b>						
14:0	4.30 ± 0.38 <sup>a</sup>	4.03 ± 0.23 <sup>a</sup>	3.98 ± 0.51 <sup>a</sup>	2.62 ± 0.34 <sup>b</sup>	2.48 ± 0.40 <sup>b</sup>	1.54 ± 0.16 <sup>c</sup>
16:0	27.1 ± 1.2 <sup>b</sup>	28.1 ± 0.2 <sup>ab</sup>	29.1 ± 0.1 <sup>a</sup>	27.1 ± 1.0 <sup>b</sup>	28.7 ± 0.9 <sup>ab</sup>	26.9 ± 1.1 <sup>b</sup>
18:0	9.10 ± 1.37 <sup>bc</sup>	8.97 ± 0.20 <sup>bc</sup>	8.28 ± 0.27 <sup>c</sup>	10.9 ± 1.2 <sup>ab</sup>	10.4 ± 1.5 <sup>b</sup>	12.7 ± 1.3 <sup>a</sup>
Total SFA	40.5 ± 1.9	41.1 ± 0.2	41.4 ± 0.4	40.6 ± 1.9	41.6 ± 0.4	41.1 ± 1.5
<b>MUFA</b>						
16:1n-7	4.49 ± 0.22 <sup>a</sup>	4.11 ± 0.11 <sup>a</sup>	4.41 ± 0.60 <sup>a</sup>	3.23 ± 0.58 <sup>b</sup>	2.48 ± 0.21 <sup>c</sup>	1.99 ± 0.42 <sup>c</sup>
18:1n-9	18.9 ± 0.9 <sup>a</sup>	18.3 ± 0.9 <sup>a</sup>	18.7 ± 0.3 <sup>a</sup>	15.5 ± 0.3 <sup>b</sup>	17.5 ± 0.9 <sup>a</sup>	15.0 ± 1.3 <sup>b</sup>
18:1n-7	3.88 ± 0.12 <sup>a</sup>	3.66 ± 0.47 <sup>ab</sup>	3.47 ± 0.28 <sup>ab</sup>	2.96 ± 0.10 <sup>bc</sup>	2.56 ± 0.24 <sup>c</sup>	2.62 ± 0.73 <sup>c</sup>
Total MUFA	27.2 ± 0.8 <sup>a</sup>	26.1 ± 0.7 <sup>a</sup>	26.6 ± 0.3 <sup>a</sup>	21.7 ± 0.3 <sup>b</sup>	22.5 ± 1.2 <sup>b</sup>	19.6 ± 2.4 <sup>c</sup>
<b>PUFA</b>						
18:2n-6 (LA)	20.9 ± 1.2 <sup>a</sup>	20.0 ± 0.8 <sup>ab</sup>	18.4 ± 0.5 <sup>bc</sup>	19.2 ± 0.3 <sup>bc</sup>	21.1 ± 1.4 <sup>a</sup>	18.2 ± 0.7 <sup>c</sup>
18:3n-3 (LLA)	1.86 ± 0.19 <sup>a</sup>	1.64 ± 0.25 <sup>ab</sup>	1.55 ± 0.31 <sup>ab</sup>	1.22 ± 0.30 <sup>bc</sup>	0.96 ± 0.25 <sup>c</sup>	0.89 ± 0.20 <sup>c</sup>
20:4n-6 (ARA)	2.91 ± 0.10 <sup>d</sup>	4.07 ± 0.62 <sup>c</sup>	3.10 ± 0.35 <sup>d</sup>	5.41 ± 0.37 <sup>b</sup>	5.52 ± 0.56 <sup>b</sup>	8.10 ± 0.57 <sup>a</sup>
20:5n-3 (EPA)	1.17 ± 0.20 <sup>bc</sup>	0.95 ± 0.20 <sup>c</sup>	1.74 ± 0.38 <sup>a</sup>	1.76 ± 0.16 <sup>a</sup>	1.63 ± 0.08 <sup>a</sup>	1.60 ± 0.39 <sup>ab</sup>
22:5n-3	1.07 ± 0.04 <sup>ab</sup>	1.05 ± 0.05 <sup>b</sup>	1.10 ± 0.12 <sup>a</sup>	1.09 ± 0.10 <sup>a</sup>	0.94 ± 0.13 <sup>b</sup>	1.30 ± 0.16 <sup>a</sup>
22:6n-3 (DHA)	4.32 ± 0.13 <sup>c</sup>	5.17 ± 0.50 <sup>bc</sup>	6.12 ± 0.10 <sup>b</sup>	8.98 ± 0.86 <sup>a</sup>	5.77 ± 0.64 <sup>bc</sup>	9.21 ± 1.67 <sup>a</sup>
Total PUFA	32.3 ± 1.4 <sup>c</sup>	32.8 ± 0.9 <sup>c</sup>	32.1 ± 0.6 <sup>c</sup>	37.7 ± 1.7 <sup>ab</sup>	35.9 ± 1.1 <sup>b</sup>	39.3 ± 1.1 <sup>a</sup>
∑n-3 PUFA	8.42 ± 0.32 <sup>c</sup>	8.82 ± 0.70 <sup>bc</sup>	10.5 ± 0.6 <sup>b</sup>	13.0 ± 1.3 <sup>a</sup>	9.31 ± 0.97 <sup>bc</sup>	13.0 ± 2.2 <sup>a</sup>
∑n-6 PUFA	23.8 ± 1.1 <sup>c</sup>	24.0 ± 0.4 <sup>c</sup>	21.5 ± 0.7 <sup>d</sup>	24.6 ± 0.5 <sup>bc</sup>	26.6 ± 1.1 <sup>a</sup>	26.3 ± 1.2 <sup>ab</sup>
∑n-3/∑n-6	0.35 ± 0.01 <sup>b</sup>	0.37 ± 0.03 <sup>b</sup>	0.49 ± 0.04 <sup>a</sup>	0.53 ± 0.05 <sup>a</sup>	0.35 ± 0.04 <sup>b</sup>	0.49 ± 0.10 <sup>a</sup>

\*Values are means of three replicates; LA, linoleic acid; LLA, linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid. <sup>a,b,c,d</sup>In each line, means with no letters or with the same letters as superscripts are not significantly different ( $P > 0.05$ ).

growth in semi-intensive systems. The reported dietary requirement for phosphorus in tilapia varies from 0.90% [30] to 0.46% [31] depending on species, fish size and diet composition. In this study, all the diets used contain sufficient amounts of phosphorus to meet this need. Since *A. filiculoides* contain no antinutrient factor, the reduction in growth of fish fed diets A<sub>30</sub>, A<sub>40</sub> and A<sub>50</sub> could be explain by the lower content of methionine and (n-3) fatty acids in the fern and thus in high AM-diets, as well as by the low digestibility of crude protein found in *A. filiculoides* [31,32]. Unfortunately, it seems that these deficiencies are not completely counterbalanced by the inputs from natural food, mainly plankton and invertebrates, through their content in methionine and other

amino acids and fatty acids.

Carcass moisture and crude protein in whole fish body were not significantly affected by dietary treatment. Conversely, lipid deposition was affected at high AM level (A<sub>50</sub>).

Several studies have been reported on the effects of substituting FM with feedstuffs on the carcass composition of fish produced. The trend obtained for moisture and crude protein were in agreement with those reported for Nile tilapia by Patel & Yakupitiyage [33] in ponds and Schneider *et al.* [34] in aquaria.

The decreasing values of lipid at high AM in diets followed the results of Pouomogne *et al.* [35] who used cocoa husks, Hossain *et al.* [36,37] who used *Sesbania*,

and Siddhuraju & Becker [38,39] who used *Mucuna* seed meal in their diets. 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 [40].

In experimental diets, percentages of myristic acid, stearic acid, palmitoleic acid, vaccenic acid, linolenic 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. Also, the increasing trend of linoleic acid (LA) and ARA levels in experimental diets is probably due to the increase of AM, which contains higher levels of that fatty acid than FM.

Because of the high content of AM in LA and ARA compared to FM, these fatty acids levels increased as AM proportion increased in diets. According to Maina *et al.* [13] quoting Olsen *et al.* [41] and Takeuchi *et al.* [42], tilapia possesses desaturation and elongation enzymes that efficiently convert C18 PUFAs to longer chain PUFAs. In experimental fish, significantly higher levels of ARA were found in fish fed high AM levels, whereas their LA showed significantly lower values. The higher values of ARA and the lower values of LA in fish compared to their levels in diets suggest *de novo* synthesis of ARA, using LA as precursor. Also, the lower values of LLA in fish compared to their levels in diets corroborated the well established fact that many freshwater fish [43], salmonids [44], rainbow trout [45] and tilapias [13,46] can readily convert LLA to EPA and DHA using metabolic pathways similar to those occurring in higher vertebrates. However, ARA, EPA, clupanodonic acid and DHA levels in all experimental fish were higher than the levels in diets. And these fatty acids values increased in AM-fed fish. According to Maina *et al.* [13] and Bahuriz & Ng [46], the fatty acid composition of many fish resembles that of their food. This was also proved in many studies in which FM or fish oil was replaced by non-conventional sources of protein or lipid [15,47-49]. Awaiss *et al.* [16] found that fatty acid composition in gudgeon and perch reflected that of the rotifer *Brachionus calyciflorus* Pallas which they were fed. In Ethiopian lakes, the differences in natural food available in several lakes resulted in differences in fatty acid composition of *O. niloticus* [50].

Moreover, many phytoplankton families [51,52], copepods and rotifers [17,53], and chironomids and oligochaetes [54] are rich sources of LLA, EPA and DHA. It seems that the low levels of the latter fatty acids in AM-diets can be compensated by the consumption of an amount of natural food from the ponds. This contribution of the natural food to fatty acid profile of fish seems to

be higher in fish fed high amounts of *Azolla* in diets. This emphasizes the need to quantify the contribution of natural food organisms to the overall nutritional budget of ponds, especially in those receiving AM. As a consequence of increase in ARA, EPA, DHA in high AM-fed fish, total PUFAs, ( $n - 3$ ) and ( $n - 6$ ) fatty acids, and  $n - 3/n - 6$  values were higher in fish fed high AM levels in ponds.

## 5. Conclusions

The present study indicated that up to 20% of AM could be incorporated in diets without adverse effects on growth in Nile tilapia, which corresponds approximately to a reduction of 30% of FM protein of the control diet. This study also showed that the incorporation of AM in diets did not affect significantly the fish carcass composition.

Replacing FM with AM in diets for Nile tilapia *O. niloticus* raised in ponds yields fish with fatty acid profiles suitable for human consumption. As a result of the contribution of natural food available in ponds, the study suggest using AM in pond to produce high quality Nile tilapia with better fatty acid profile for human health.

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