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Comparability of amino acids composition in leaves of *Azolla filiculoides, Moringa oleifera* and *Dialum guineensis* as sources of proteins in food of fish

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ABSTRACT: The amino acid compositions of *Azolla filiculoides, Moringa oleifera* and *Dialum guineensis* were determined on a dry weight basis. The total amino acids ranged from 50.83-83.01g/100 g crude protein and the essential total percentage of amino acids was 42.16% (*A. filiculoides*), 45.64% (*M. oleifera*) and 45.73% (*D. guineensis*). Acid amino acids (aspartic acid and glutamic acid) were the most concentrated amino acids in the samples while aromatic amino acids were slightly concentrated. In all the amino acids determined, *D. guineensis* had the most concentrated amino acids than *A. filiculoides* and *M. oleifera*. The calculated isoelectric points were 1.7 (*A. filiculoides*), 1.7 (*M. oleifera*) and 2.8 (*D. guineensis*), showing they can all be precipitated at acidic pH. Methionine was the limiting amino acid in *A. filiculoides.* The amino acid score showed that lysine ranged from 0.44-0.94 (on provisional essential amino acid scoring pattern) translating the quality of the sources of studied proteins. The predicted protein efficiency ratio (2.70-3.81) showed that the quality of protein was high while the essential amino acid index range was 0-1.09.

The aim of this work was to compare the composition in amino acids of the samples. *A. filiculoides, M. oleifera* and *D. guineensis* can be recommended in the food of fish taking into account their food values but must be employed in limited proportions.

KEYWORDS: Isoelectric point, amino acid score, proportion.

1 INTRODUCTION

In fish breeding, the major constraint determining the production cost is the food. This food consists of a raw material association whose principal one is the fish meal. However the fish meal, currently available in Benin, is not only of doubtful quality but also has a high cost. It is thus significant to find alternative sources of proteins to the fish meal in order to reduce this raw material dependence of the halieutics and the anthropic pressure on our water levels.

More and more, the sources of plant proteins are developed in the food of fish [1]. These plants, rich in proteins, are not inevitably of good quality (essential amino acids…) in order to allow good performances of fish growth.

Azolla is an ideal feed substitute, it is an aquatic fern (pteridophyte), floating on water surface of flooded rice fields, small ponds ,and canal. Its size is 1-5cm except for a giant Azolla nilotica (which size is 15cm approximately). Seven extant azolla species are recognized (some taxonomists recognized 6 species), are distributions widely from temperature to tropical regions [2]. Azolla has been used as a feed for pig, duck, and fish. [3] reported that, azolla has high protein content (20-30% on dry weight basis) rich in almost all essential amino acids, vitamin A, vitamin B-complex, beta-carotene and minerals Calcium, phosphorus, potassium, iron, copper and magnesium).

Moringa oleifera Lam., a member of the family Moringaceae, is a fast-growing plant widely available in the tropics and subtropics with great economic importance for the food and medical industry [4]. The seeds are a rich oil and protein source and can also be used for the purification of water. The roots are a source of spices. The leaves are rich in vitamins, minerals, carotenoids, ascorbic acid and iron [5].

From Fabaceae family and under the family of Caesalpinaceae, *Dialum guineensis* has its sheets made up of stem common, long 5.13cm with an odd final leaflet and generally two pairs of folder opposite or alternate [6]. Its sheets are used in traditional pharmacopeia for the treatment of various diseases, the consumption of the fruits, its branches are used as traditional technique of fishing (acadja) and firewood [7].

The aim of this experiment is to analyze chemical composition of *Azolla filiculoides*, *Moringa oleifera* and *Dialum guineensis* meals so as to provide information which will help in incorporating any of these unconventional sources into fish feed ingredients during the feed formulation by fish nutritionist and fish farmers who may want to use them as on-feed ingredients.

2 MATERIALS AND METHODS

2.1 SAMPLES COLLECTION AND FLOURS PRODUCTION

The fresh leaves of *Moringa oleifera* (MM) and *Dialum guineensis* (DM) were collected at Akassato (Benin) while *Azolla filiculoides* (AM) was collected from some ponds on site of Research Laboratory in Wet Lands of the University of Abomey-Calavi. They were collected and used to produce flours according to the method [8].

2.2 CHEMICAL ANALYSES

Crud protein, crud lipid, dry matter and ash in the flours produced were analyzed following [9] procedures. The amino acids of the flours were analyzed with a Waters HPLC system (Waters 474, Waters, Milford, MA, USA) including two pumps (Model 515, Waters), an auto sampler (Model 717, Waters), a fluorescence detector (Model 474, Waters) and a temperature control module. These amino acids analyses were done following the method previously described by [10]. Aminobutyric acid was added as an internal standard before hydrolyzation. Amino acids were derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and then separated with a C-18 reverse-phase column Waters Acc. Tag (150 mm × 3.9 mm). All of these analyses were conducted in the Laboratory of Aquatic Animal Nutrition of Kagoshima University (Japan).

2.3 ESTIMATION OF ISOELECTRIC POINT (PI), QUALITY OF DIETARY PROTEIN AND PREDICTED PROTEIN EFFICIENCY RATIO (P-PER)

The predicted isoelectric point was evaluated according to [11]

$$\text{plm} = \sum_{i=1}^{n=1} \text{pliXi}$$

Where: plm = The isoelectric point of the mixture of amino acid, pli = The isoelectric point of the ith amino acids in the mixture, Xi= The mass or mole fraction of the amino acids in the mixture.

The quality of dietary protein was estimated by Amino acid score (AMSS) based on provisional amino acid scoring pattern [12]

$$\text{AMSS} = \frac{mg\ of\ amino\ in\ 1g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1g\ reference\ protein} \times \frac{100}{1}$$

The Predicted Protein Efficiency Ratio (P-PER) of the fresh sample was calculated from their amino acid composition based on the equation developed by [13]:

P-PER = 0.468+0.454 (Leu) – 0.105 (Tyr)

Essential amino acid index (EAA Index) was calculated by using the ratio of test protein to the reference protein for each of the eight essential amino acids plus histidine in the equation [14]:

$$\text{EAA Index} = \sqrt[9]{\frac{mg\ lysine\ in\ 1\ g\ test\ protein}{mg\ lysine\ in\ 1\ g\ reference\ protein} \times etc.\ for\ all\ 8\ essential\ amino\ acids + His}$$

2.4 STATISTICAL ANALYSIS

The statistical analysis carried out included the determination of the grand mean, standard deviation (SD) and the coefficients of variation percent (CV %).

3 RESULTS AND DISCUSSION

In table 1, the data indicate that AM and MM meal were rich in quality crude protein (27 and 27.7% respectively) than DM (22.05%) whereas low levels of crude fats were obtained for DM, AM and MM meals respectively. Analysis results for protein, amino acid and fat contents in AM and MM were different from those reported by [15], [16] and [17] probably due to differences in either the analysis procedures employed or the methods of production of AM and MM meals.

Table 1: Proximate composition (%) of Azolla filiculoides, Moringa oleifera and Dialum guineensis (dry weight)

Parameters	AM	MM	DM
Dry matter	13.93	10.29	14.12
Crude protein	27.00	27.70	22.05
Crude fat	4.31	6.54	3.99
Ash	15.72	12.96	18.66

Table 2: Amino acid composition (g/100g crude protein) of Azolla filiculoides, Moringa oleifera and Dialum guineensis (dry weight)

Amino acids (g/100g)	Concentration			Mean	SD	CV %
	AM	MM	DM			
Hydroxyproline	0.25	0.18	1.63	0.68	0.81	119.08
Aspatic Acid	6.18	5.48	11	7.55	3.00	39.78
Threonine[a]	2.48	2.23	3.21	2.64	0.50	19.28
Serine	3.44	2.92	4.22	3.52	0.65	18.55
Glutamic Acid	8.88	7.58	9.38	8.61	0.92	10.78
Proline	2.96	2.92	5.85	3.91	1.68	42.97
Glycine	2.96	2.74	3.58	3.09	0.43	14.08
Alanine	3.96	3.07	4.22	3.75	0.60	16.08
Valine[a]	1.96	2.05	3.58	2.53	0.91	35.98
Methionine[a]	0.00	0.86	1.32	0.72	0.67	92.20
Isoleucine[a]	1.22	1.37	2.27	1.62	0.56	35.05
Leucine[a]	4.18	4.29	6.17	4.88	1.11	22.92
Tyrosine	3.44	2.74	5.17	3.78	1.25	33.06
Phenylalanine[a]	3.44	3.61	6.17	4.40	1.52	34.70
Histidine[a]	1.74	1.55	2.90	2.06	0.73	35.41
Tryptophan[a]	1.48	1.04	1.32	1.28	0.22	17.39
Lysine[a]	2.70	2.41	5.17	3.42	1.51	44.26
Arginine[a]	4.18	3.79	5.85	4.60	1.09	23.75

[a]Essential amino acid (EAA); mean value is grand mean from the mean values of the amino acids.

In most of the results on pair wise basis, the values of amino acids for the *Dialum guineensis* meal (DM) were all better than the values of amino acids of *Moringa oleifera* and *Azolla filiculoides* (AM) meals samples (table 2). On the other hand, levels of Valine, Méthionine, Isoleucine, Leucine and Phenylalanine in MM meal were correspondingly higher than those in AM meal, meaning that *M. oleifera* meal (MM) was 27.78% best in 18 parameters (5/18) of the amino acids than in *A. filiculoides* meal, rich in amino acids with 72.22% (13/18). Similar trend between raw and germinated wheat flours amino acid profile have also been observed [18], between maggot, earthworm and soybean meals [19], between peper, onion, garlic, ginger and tomato [20].

Leucine and phenylalanine were the highest concentrated EAA (6.17g/100g cp) in *D.guineensis* meal, tryptophan was the most concentrated EAA in *A. filiculoides* meal (1.48 g/100g cp) but none methionine in *Azolla filliculoides* (0.00 g/100g cp). Similar observations were also reported by [21] and [19] in which leucine was the most concentrated EAA in raw wheat and millet samples and maggot meal respectively. The zero value of methionine in *A. filiculoides* limits its use in the fish feed without external contribution of methionine synthesized or ingredient rich in methionine.

In the generally, the values of amino acids essential in these plants (Table 2) answer in 60% of the cases with the requirements in amino acids for fish for breeding like the tilapia and the catfish. The methionine and isoleucine, defective in the whole of the plants (0-1.32 and 1.22-2.27g/100g cp respectively), are largely lower than the requirements out of methionine and isoleucine (2.3-4g/100g cp) for the majority for fish for breeding especially for Tilapia and catfish [22].The same observation is made with the lysine rate (2.70 and 2.41g/100g cp at AM and MM) except DM where the lysine rate (5.17g/100g cp) meets the requirements out of lysine (4.8-6.1g/100g cp) for fish for breeding like the tilapia and the catfish [22]. As for the arginine, its rate in the various sources of plant proteins (3.79-5.85g/100g cp) is located in the range of needs for fish of breeding [22] but the rates of arginine in AM and MM are in on this side needs for the fish tilapia and catfish (4.2 and 4.3g/100g cp respectively).These data proved us that AM, MM and DM can be well developed in the fish food with animal source of protein rich in essential amino acids.

The most concentrated amino acids in DM were aspatic acid and glutamic acid (11g/100g and 9.38 g/100g cp respectively). These values corroborate [23] which showed that *Cucurbita maxima*, *Amaranthus viridis* and *Basella alba* are rich in these acid amino acids. These amino acids were the least concentrated in MM (7.58g/100g and 5.48 g/100g cp respectively) than AM while its essential amino acids except threonine, tryptophan and basic amino acid was more concentrated in MM. These values compared favourably with the values reported for maggot, earthworm and soybean meal [19]. The Lys/Arg values ranged as follows: 0.65 (AM), 0.64 (MM) and 0.88 (DM). In the all samples (Table 2), the level of Lysine was twice less than the level of Arginine in each sample. In homeotherms, the well-known examples are antagonisms arising from dietary imbalances of lysine-arginine. No convincing evidence exists, however, for lysine-arginine antagonism in fish [22].

Nutritive value of protein depends on its capacity to satisfy the needs for nitrogen and essential amino acids [24]. Table 3 shows several quality parameters of protein in the samples. The EAA ranged between 23.2g/100g cp to 37.96g/100g cp. These values were far from the values of 56.6g/100g cp of the egg reference protein [25] but better than 19.0g/100g cp for *Colocynthis citrulus* flours [26]; 21.5g/100g cp in sorghum grains [27]. The values of EAA in AM and MM were slightly higher than 21.0-23.4g/100g cp obtained in millet grains [21] but values of EAA in DM was comparably close to the values reported for maggot and earthworm meals (31.4-34.5g/100g cp) [19].

The percentages of EAA/TAA (Table 3) for the three samples (42.16(AM), 45.64(MM) and 45.73(DM)) could be favorably compared with that of beach pea protein isolates (43.8-44.4%) [28] but were lower than those reported for *Cucurbita maxima* (53.4%), *Amaranthus viridis* (50%), *Basella alba* (51.3%) [23] and maggot meal, earthworm meal, soybean meal [19].

The P-PER is one of the quality parameters used for protein evaluation [29]. Its values in this report ranged from 2.70 in *M. oleifera* meal to 3.81 in *D. guineensis* meal. These results showed the physiological utility in the body of DM which would be much better than AM and MM. On the average, the three would be much better than the 2.55 in fermented cocoa nibs [30], 1.32 to 1.66 in the pearl millet grains [21] but were favorably compared for *Pterocarpus mildbraedii* (2.92) [31].

Table 3: EAA, Non EAA, Acidic, Basic, Sulphur and Aromatic Acid Contents (g/100g cp) of Azolla filiculoides, Moringa oleifera and Dialum guineensis (dry weight)

Amino acids (g/100g crude protein)	Concentration			Mean	SD	CV %
	AM	MM	DM			
TAA	55.45	50.83	83.01	63.10	17.40	27.58
TEAA*	23.38	23.2	37.96	28.18	8.47	30.06
without His	21.64	21.65	35.06	26.12	7.75	29.66
%TEAA	42.16	45.64	45.73	44.51	2.04	4.57
without His	39.03	42.59	42.24	41.29	1.96	4.75
TNEAA	32.07	27.63	45.05	34.92	9.05	25.93
%TNEAA	57.84	54.36	54.27	55.49	2.04	3.67
TAAA	15.06	13.06	20.38	16.17	3.78	23.40
%TAAA	27.16	25.69	24.55	25.80	1.31	5.07
TBAA	8.62	7.75	13.92	10.10	3.34	33.08
%TBAA	15.55	15.25	16.77	15.86	0.81	5.08
TSAA*	0	0.86	1.32	0.73	0.67	92.21
%TSAA	0	1.69	1.59	1.09	0.95	86.72
TArAA	6.88	6.35	11.34	8.19	2.74	33.47
%TArAA	12.41	12.49	13.66	12.85	0.70	5.44
Lys/Arg	0.65	0.64	0.88	0.72	0.14	18.77
Leu-Ile	2.96	2.92	3.9	3.26	0.55	17.01
P-PER	2.73	2.70	3.81	3.08	0.63	20.53
pI	1.7	1.7	2.8	2.07	0.64	30.73
EAA Index	0	0.64	1.09	0.58	0.55	94.99

*Total amino acid (TAA); Total essential amino acid (TEAA); Total non-essential amino acid (TNEAA); Total acidic amino acid (TAAA); Total basic amino acid (TBAA); total sulphur amino acid (TSAA); *without cysteine*

The experimentally determined P-PER usually range from 0.0 for a very poor protein to a maximum possible of just over 4 [32]. These values showed that these sources of plants proteins are good quality and higher than the reported P-PER values of some legume flours/concentrates and fish species: *Prosopis africana* (2.3) [33], *Lathynis sativus* L. (1.03) [34], *Phaseolus coccineus* (1.91) [35], *Clarias anguillaris* (2.22), *Oreochromis niloticus* (1.92) and *Cynoglossus senegalensis* (1.89) [36].

The calculated isoelectric point (pI) ranged from 1.7 (*A. filiculoides* and *M. oleifera*) to 2.8 (*D. guineensis*). These values are very low compared to those obtained by [37] and [38] on *Numida meleagris* (5.48-5.69) and *Amaranthus hybridus* (4.2) respectively but are comparable with those obtained on *Telfairia occidentalis* (2.8) [38]. The calculation of pI from the AA would assist in the production of the protein isolate of an organic product. The information on pI is a good starting point in predicting the pI for proteins in order to enhance a quick precipitation of protein isolate from biological samples [15]. The low values of pI could be a function of the TAAA (24.55-27.16%) which were much higher than the TBAA (15.25-16.77%) (Table 3).

The essential amino acid index (EAA Index) ranged from 0 to 1.09. EAAI is useful as a rapid tool to evaluate food formulations for protein quality, although it does not account for differences in protein quality due to various processing methods or certain chemical reactions [40].

In Table 4, the content of some essential amino acids in all plants was lower than [29] except tryptophan but also in phenylalanine at DM.

Table 4: Amino acid score of A. filiculoides, M. oleifera and D. guineensis based on provisional essential amino acid scoring pattern

AMSS / EAA	AM	MM	DM
Threonine	0.62	0.56	0.80
Valine	0.39	0.41	0.72
Methionine	0	0.25	0.38
Isoleucine	0.31	0.34	0.57
Leucine	0.60	0.61	0.88
Phenylalanine	0.57	0.60	1.03
Histidine	-	-	-
Tryptophan	1.48	1.04	1.32
Lysine	0.49	0.44	0.94
Arginine	-	-	-

- : not determinated

The amino acid content of the plants is low, except tryptophan, which is close to the recommended value. The results of amino acid score revealed that the essential amino acid scores are lower than one, except tryptophan with 1.04-1.48 mg/g values and phenylalanine in DM (1.03 mg/g).

4 CONCLUSIONS

The present study indicates that *Azolla filiculoides*, *Moringa oleifera* and *Dialum guineensis* were rich in crude protein but also in certain amino essential although for the majority, they are weak compared to essential amino acids based on the FAO/WHO provisional pattern. Nevertheless, they can be well developed in the fish food like sources complementary to proteins in combination with other sources of animal proteins.

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