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Preliminary assessment of water spinach (*Ipomoea aquatica*) and morning glory (*Ipomoea asarifolia)* leaves meals as non-conventional fish feed stuffs

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Abstract
Assessment of nutritional qualities of aquatic weeds grown in Kainji Lake, Nigeria; Water spinach (*Ipomoea aquatica*) and morning glory (*Ipomoea asarifolia*) leaf-meals was carried out using standard laboratory methods. The nutritive values, mineralization and protein building units for the aquatic weeds were determined as potential fish feedstuffs. The leaf meal of *I. aquatica* contained crude ash (12.50%), crude fibre (7.62%), crude protein (25.60%), crude lipid (6.33%), Carbohydrate (47.95%), metabolizable energy value of 311.24kcal/g and energy/protein ratio of 12.16. While the nutritive values in *I. asarifolia* leaf-meal were crude ash (11.50%), crude fibre (8.77%), crude protein (21.90%), crude lipid (5.88%) and carbohydrate (48.05%), metabolizable energy (294.81kcal/g) and energy/protein ratio (13.46). The mineral contents of the leaf-meals of the two species of the Ipomoea were potassium (444 mg/100g), calcium (163mg/100g), Sodium (159.8 mg/100g), phosphorus (86 mg/100g), magnesium (52 mg/100g), copper (5.3 mg/100 g), zinc (4.1 mg/100g), iron (3.2 mg/100g) and manganese (2.3 mg/100g). *I. aquatic* leaf meal contained 407 mg/100g and 26 mg/100g higher in both indispensable and dispensable amino acids values than *I. asarifolia* leaf meal. The two aquatic leaves had abundant components of essential amino acids. Empirically, the *Ipomoea spp* exhibited or showed low methionine contents which are a common occurence in plant protein. *I. aquatic* and *I. asarifolia* leaf meals could be good supplements for some nutrients and natural antioxidants as an alternative for fish feed ingredients. Therefore, such feed can an alternative meal for *Clariids* species and cultured cichlids.

Keywords: *Ipomoea aquatica, Ipomoea asarifolia,* proximate analysis, minerals and amino acids

1. Introduction
Fish feed generally constitutes 60-70% of the operational cost in intensive and semi-intensive aquaculture system (Singh *et al*., 2006) [21]. The fish feed used in aquaculture is quite expensive, irregular and scarce in supply in many third world countries. These feeds are sometimes adulterated, contaminated with pathogen as well as containing harmful chemicals for human health. Naturally, there is a need for the development of healthy, hygienic fish feed which influences positively the growth and quality of the cultured fish. Considering the importance of nutritionally balanced and cost-effective alternative diets for fish, there is a need for research effort to evaluate the nutritive value of different non-conventional feed resources, including terrestrial and aquatic macrophytes (Edwards *et al*., 1985; Wikipedia, 2005; Mundal and Ray, 1999; Kalita *et al*., 2006) [9, 27, 15].

Aquatic and terrestrial macrophytes have been used as supplementary feeds in fish farming since the early times of freshwater fish culture (Bardach *et al.*, 1972) [6] and still play an important role as fish feed in extensive culture systems (Edwards, 1987) [8]. The aquatic weeds have been shown to contain substantial amounts of protein and minerals (Ray and Das, 1995) [20]. Valente *et al*., (2006) [26], reported clearly that macro algae such as *G. bursapastoris*, *U. rigida*and *G. cornea*have great potential as alternative ingredients in diets for European sea bass juveniles at dietary inclusion levels up to 10% with no adverse effects on growth performance and feed utilization efficiency. There is high competition for the same foodstuffs between man and domestic animals. For both economic and practical reasons, fish feed should be prepared using locally available protein sources, preferably from those unsuitable for human consumption (Bag *et al.*, 2012) [5]. It is therefore, very crucial to find an alternative to reduce feeding cost, and to make aquaculture a viable and attractive venture. Mukherjee *et al.*, (2010) [16] reinforced the utilization of some aquatic weeds as promising sources of nutrients in

fish-feed. Water spinach is a good source of protein and can be used as feed for all kinds of animal and for humans. The foliage contains protein in the range of 23.6% in the dry season and 27.6% in the wet season (Oshodi *et al*., 1999) [19], and is also a good source of trace minerals (mg/kg): Zn, 5.03; Mn, 22.2; Cu, 1.37 and Fe, 75.3 (Yoshimura *et al*., 1991; USDA, 2000) [28, 25] and rich in vitamin A and C. Tomori and Obijole, (2000) [24] reported that the mineral element contents in the leaves were high, in particular the concentration of K and Fe. Also the leaves contain moderate concentrations of Na, Ca, Mg and P, with low Cu, Mn and Zn contents. Water spinach is usually consumed by both man and animals, readily available as marginal and emergent weeds. *Ipomoea asarifolia* is a species of *Ipomoea* morning glory. The species belong to the *Convolvulaceae* family and is an annual herb. The objective of this experiment is to evaluate or assess the nutritional qualities of water spinach (*Ipomoea aquatica*) and the morning glory (*Ipomoea asarifolia*) leaves as an alternative fish feed ingredients.

2. Materials and Methods

2.1 Samples Collection and Treatments

Samples of water spinach and morning glory used in this study were collected along the bank of river Niger at Monnai, a village near New-Bussa in Niger State Nigeria. Prior to analysis, the plant leaves were separated from the stalk and washed thoroughly with distilled water. The residual moisture was evaporated at room temperature (37 ^{o}C). The leaves were sun-dried for 48hours until constant weight was obtained (Fasakin, 2004) [12]. The dried leaves were then grounded in a wooden mortar, sieved through a fined mesh-sized and stored in polyethylene bags. The powdered samples were used for both proximate and mineral analysis. Moisture content was however evaluated with the usage of fresh leaves.

2.2 Proximate analysis

2.2.1 Moisture Content

Moisture content of *Ipomoea aquatic* and *Ipomoea asarifolia* were determined by drying the leaves (in triplicate) in a Gallenkamp oven at 105 ^{o}C until constant weight was attended (AOAC, 1990) [2].

2.2.2 Ash Content

Ash content was determined by drying ash in Lenton muffle furnace at 525 ^{o}C for 24hours.

2.2.3 Crude Protein Content

Crude protein content was calculated by multiplying the values obtained from Kjeldahl's nitrogen by a protein factor 5.3, a factor recommended for vegetable analysis (Bernice and Merril, 1975) [7].

2.2.4 Crude lipid

Crude lipid was quantified by the method described by (AOAC, 1990) [2] using the solvent apparatus and n-hexane as a solvent.

2.2.5 Crude fibre

Crude fibre was estimated by acid-based digestion with 1.25% H_2SO_4 (w/v) and 1.25% NaOH (w/v) solutions (AOAC, 1990) [2].

2.2.6 Carbohydrates

Available carbohydrates were calculated by difference (i.e., total sum of crude protein, crude, fibre, crude, ash, and crude lipid deducted from 100% DM (AOAC, 1990) [2].

The sample calorific value was estimated (in kcal) according to the formula:

Energy = (g protein x 2.44) + (g lipid x 8.37) + (g available carbohydrate x3.57) (Asibey-Berko and Taye, 1999) [3].

2.3 Mineral analysis

2.3.1 Sample digestion

One gram powdered sample was put in digestion flask followed by addition of 25.0cm^3 concentrated HNO_3. The flask was then heated in Tecator digestion block until evolution of brown fume stopped. 1cm^3 of Perchloric acid was added to the mixture and the content was further heated to a clear solution. After heating, 30cm^3 of hot distilled water was added to the digest and heated to boiling. The solution was then filtered hot into a clean 50cm^3 volumetric flask, cooled and made up to the mark with distilled water (Tayie, and Asibey-Berko 2001) [23].

2.4 Mineral quantification

The concentration of calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and zinc (Zn) etc in the digest were performed with as Alpha-4 model atomic absorption spectrophotometer with standard air-acetylene flame. The sodium (Na) and potassium (K) content was analyzed by flame atomic emission spectrophotometry using corning 400 spectrophotometer. Phosphorus (P) content was analysed with Jenway 6100 spectrophotometer using ammonium vanadate-molybdate colorimeter method (AOAC, 1990) [2]. For calcium (Ca), magnesium (Mg) determination, in order to avoid potential anionic interferences, 4cm^3 of 5% lanthanum chloride ($LaCl_27H_2O$) solution was added to 1 cm^3 of the digest in a 50cm^3 volumetric flask and solution made up to the mark with distill water (Amaro-Loper *et al.*, 1999) [1].

3. Results

3.1 Nutritional value

The nutritional analysis of these two species of the *Ipomoea* is presented in Table 1. The result of proximate composition of *Ipomoea africana* and *Ipomoea asarifolia* leaf-meals revealed that moisture contents were 4.75% in *I. africana* leaf-meal and 5.32% in *I. asarifolia* leaf-meal. The dietary composition analysis of the leaf-meals of these two species of the *Ipomoea* were crude ash (12.50%), crude fibre (7.62%), crude protein (25.60%), crude lipid (6.33%), carbohydrate (47.95%) metabolizable energy (311.24kcal/g) and energy/protein ratio (12.16) respectively in *I. africana* leaf-meal. The nutritive values in *I. asarifolia* leaf-meal were crude ash (11.50%), crude fibre (8.77%), crude protein (21.90%), crude lipid (5.88%), carbohydrate (48.05%), metabolizable energy (294.81kcal/g) and energy/protein ratio (13.46) respectively in *I. africana* leaf-meal (Table 1).

Table 1: Average Mean Values of Proximate Composition in *Ipomoea aquatica* and *Ipomoea asarifolia* leaf-meals

Parameters	Concentration (%) Dry Weight	
	***Ipomoea aquatic* (Water spinach) leaf meal**	***Ipomoea asarifolia* (Morning glory) leaf meal**
Moisture content (%)	4.75	5.32
Crude Ash (%)	12.50	11.50
Crude fibre (%)	7.62	8.77
Crude protein (%)	25.60	21.90
Lipid (%)	6.33	5.88
Carbohydrate (%)	47.95	48.05
Calculated ME (kcal/100g)	311.24	294.81
Energy/ protein ratio	12.16	13.46

*Nitrogen Free Extract (NFE) is calculated by difference = 100 – (protein + lipid + fibre + ash)
**Metabolizable energy was calculated using Atwater's calculation as described by Smith (1983), where 1g crude protein (CP), Lipid (EE) and NFE (Carbohydrate) yields 3.5, 8.5 and 3.5 kcal/g respectively.

3.2 Mineral Contents

Mean values for mineral contents of nutritional importance are presented in Table 2. Potassium (444mg/100g and 440mg/100g) was the largest macronutrient element, followed by calcium (163mg/100g and 161mg/100g), sodium (159mg/100g and 152mg/100g), phosphorus (86mg/100g and 78mg/100g) and magnesium (52 mg/100g and 48g/100g). Copper (5.3mg/100g and 4.2mg/100g) was the predominant micronutrient element, followed by zinc (4.1mg/100g and 3.98mg/100g), iron (3.2mg/100g and 2.0mg/100g) and manganese (2.3±0.01 mg/100g and 2.10mg/100g) (Table 2).

Table 2: Average Mean Values of Mineral Contents in *Ipomoea aquatic* and *Ipomoea asarifolia's* leaf-meals

Parameters	***Ipomoea aquatic* (Water spinach) leaf meal**	***Ipomoea asarifolia* (Morning glory) leaf meal**
Potassium (K)	444.00	440.00
Sodium (Na)	159.00	152.00
Calcium (Ca)	163.00	161.00
Magnesium (Mg)	52.00	48.00
Phosphorus (P)	86.00	78.00
Copper (Cu)	5.30	4.20
Iron (Fe)	3.20	2.00
Manganese (Mn)	2.30	2.10
Zinc (Zn)	4.10	3.89
Cobalt (Co)	0.04	0.02
K/Na	2.79	2.89
Ca/P	1.90	2.06

3.3 Amino Acids Contents

The composition and amount of amino acids in *I. aquatic* and *I. asarifolia* leaf meals are presented in Table 3. The *I. aquatica* contained high amount of essential amino acids (6584mg/100g) and non-essential amino acids (10974mg/100g) representing a total amino acids content of 17558mg/100g, and also, *I. asarifolia* leaf meal also high amount of essential amino acids (5177mg/100g) and non-essential amino acids (10948mg/100g) representing a total amino acids content of 16125mg/100g. The most abundant components of essential amino acids were leucine (1365mg/100g and 1355mg/100g), Tyrosine + phenylalanine (1124mg/100g and 1122mg/100g), lysine (682mg/100g and 680mg/100g) and threonine (606 mg/100g and 600mg/100g) (Table 3).

Table 3: Amino acids profile of *Ipomoea aquatica*, and *Ipomoea asarifolia* leaf-meals (dry weight basis in mg/100g)

Parameters	***Ipomoea aquatic* (Water spinach) leaf-meal**	***Ipomoea asarifolia* (Morning glory) leaf-meal**
Essential (Indispensable)		
Threonine (Thr)	606	600
Methionine (Met)	145	140
Iso-leucine (Ile)	495	489
Leucine (Leu)	1365	1355
Tyrosine (Tyr)	345	340
Phenylalanine (Phe)	779	773
Tyrosine + phenylalanine (Tyr + Phe)	1124	1122
Lysine (Lys)	682	680
Histidine (His)	348	345
Arginine (Arg)	695	688
Total	6584	5177
Non- Essential (Dispensable)		
Aspartic acid |(Asp)	2335	2328
Serine (Ser)	365	360
Glutamic acid (Glu)	1364	1360
Glycine (Gly)	4735	4735
Alanine (Ala)	1250	1245

Proleine (Pro)	925	920
Total	10974	10948
Total Amino Acids	17,558	16125

Values are means (± SD) of triplicate analysis
*Source: FAO/WHO/UNU, 2007.

4. Discussion

The proximate compositions of these two species of *Ipomoea* presented in Table 1 revealed that *Ipomoea africana* and *Ipomoea asarifolia* leaf-meals had moisture content of 4.75% and 5.32% respectively. The nutritive value in *I. africana* leaf-meal were crude ash (12.50%), crude fibre (7.62%), crude protein (25.60%), crude lipid (6.33%), carbohydrate (47.95%) and those of *I. asarifolia* leaf-meal were crude ash (11.50%), crude fibre (8.77%), crude protein (21.90%), crude lipid (5.88%), and carbohydrate (48.05%). The results of these leaf meals determined were lower than those reported for *I. aquatica* and *I. asarifolia* leaf-meals grown in other countries like Vietnamese (Ogle *et al.*, 2001) [18] and Nigeria (Okayi and Abe, 2003) as well as of *I. asarifolia* leaves (Asibey- Berko and Tayie, 1999; Ishida *et al.*, 2000) [3]. The ash content (1.6%) was comparable to the reported value for the leaf-meals of *I. asarifolia* leaves (1.8%) by Asibey-Berko and Taiye (1999) [3], but lower than values recorded for Vietnamese *I. aquatica* leaves (14.44%) recorded by Ogle *et al.*, 2001) and those from Swaziland (17.87%) Ogle and Grivetti, (1985). Gross energy value (141.4kcal/g) was also lower than those recorded by Nigerian *I. aquatica* leaves (300kcal/g) and *I. asarifolia* leaves (238.3kcal/g) (Asibey-Berko and Tayie, 1999) [3].

These macronutrient element values were lower than those obtained in Nigerian *I. aquatica* leaves (Okayi and Abe, 2003) and *I. asarifolia* leave-meals (Asibey- Berko and Tayie, (1999). The concentrations of copper and zinc were higher, as compared with that found in Nigerian *I. aquatica* leaves (5.30 and 4.10mg/100g) respectively (Okayi and Abe, 2003). The iron content was extremely lower when compared to values 210.30 and 16.67mg/100g reported from *I. aquatica* leaves grown in other geographical regions (Ogle *et al.*, 2001, Meng, 2003) and those from *I. asarifolia* leave-meals (36.69-147.87mg/100g) recorded by Taiye and Asibey-Berko, 1999) [3]. The leave-meals contained high amounts of the amino acids lysine and tryptophan, which are lacking in cereals, hence, can complement the cereal-based staple diets in the vicinity.

Moreover, these species have the potential to be a resource for supplementation of some essential amino acids (45.5%) as it was represented in leucine, 45% in Tyrosine + Phenylalanine and 40.4% in threonine of the Recommended Dietary Allowances (RDA) for the essential amino acid uptake of adults, considering a 100g se rving size for a 18–30year-old male of 70kg (FAO/WHO/UNU. 1985).

5. Conclusions

The assessed *Ipomoea aquatica* and *Ipomoea asarifolia* leave meals revealed that, the plants contained an appreciable amount nutrients, minerals and essential amino acids. Therefore, the usage of these plants could be substituted as dietary ingredients in fish nutrition such as *Clariids* species and cultured cichlids. Thus, this study may provide valuable information on the possible use of *I. aquatica* and the *I. asarifolia* leaf-meals in the nutritional and feed formulation industries.

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