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Evaluation of the nutritional status of *Nymphaea lotus* L. and *Pistia stratiotes* L. shoots in relation to their utilization as fish and animal feed

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Abstract

The present work aimed to find a new natural food resource suitable to reduce the cost of commercial feeds, through evaluating the nutritional potential of two commonly available, aquatic weeds; *Pistia stratiotes* L and *Nymphaea lotus* L from El-Serw irrigation canal, which situated at North Eastern part of the Nile Delta South of Manzalah Lake. The organic and inorganic contents were estimated, in addition to the phytochemical screening for some biologically active substances. From the obtained results, *N. lotus* stem was shown to have the highest values of the crude and digestible proteins, growth energy, protein to energy, protein to lipid and protein to carbohydrates ratios. The recovery of amino acids from acid hydrolysis revealed the presence of fifteen amino acids in the three different samples but with different concentrations. GLC analysis of fatty acids indicate the presence of different numbers and concentrations of fatty acids, in which linolenic acid (an omega -3 fatty acid) represents the major constituent of *N. lotus* stems fatty acids**,** while *N. lotus* leaves and *P. stratiotes* shoots are rich in linoleic acid (an omega-6 fatty acid). In addition, the chemical analysis of the studied species showed a considerable difference ($P < 0.05$) in their mineral composition. Besides, total phenolic content ranged between 2.7 and 2.4 % and phytochemical screening revealed the presence of flavonoides, alkaloides, tannins, sterols, chlorides and sulphates in the two species. The present study demonstrates that, the commercial exploitation of these aquatic weeds, particularly *N. lotus* stem and leaves, as cost-effective and balanced artificial fish and animal feeds appear to be promising.

Keywords: Evaluation, nutritional status, Nymphaea lotus L., Pistia stratiotes L, utilization, fish, animal feed.

1. Introduction

Aquatic plants grow profusely in lakes and waterways all-over the world and have in recent decades their negative effects magnified by man's intensive use of natural water bodies. Eradication of the weeds has proved almost impossible and even reasonable control is difficult. Turning these weeds to productive use would be desirable if it would partly offset the costs involved in mechanical removal. Among other uses, there has been considerable interest in using aquatic plants as a source of animal feed (Anon, 1984; Abulude, 2005; Haroon, 2008; Sharshar and Haroon, 2009 and Shaltout *et al*., 2010).

From the aquatic vegetation in the Nile Delta region two plant species representing free floating hydrophytes *Pistia stratiotes* L. and floating leaves *Nymphaea lotus* L. had been selected for the present investigation as a complementary study of how these plants affect on the surrounding media and their role in the microbial community structure (Haroon and Daboor, 2009). *P. stratiotes* is a free- floating stoloniferous herb commonly found in ponds and streams. Its leaves are obovate, light green in colour and have many prominent longitudinal veins (Arber, 1991). According to (Susanne and Zhang, 2003) the plant was recorded in the Americas (North Carolina to Argentina), Africa (Egypt to the Cape), India, and Southeast Asia to northeastern Australia. In Egypt *P. stratiotes* L. occurs as a dominant species in the Nile Delta, the River Nile system north wards, including northern lakes but not further south in the River Nile section at Aswan (Zahran and Willis, 2003). *P. stratiotes* L was formely restricted in Egypt to Fariskur in the Nile Delta. It has recently been recorded from several localities in the Canals of the northern regions of the Nile Delta, also reaching Embaba, near Cairo (Boulos, 2005). *N. lotus* is a perennial or rarely annual herbaceous aquatic plant, whose leaves are floated or submerged in water. It is a good phtyto-accumulator and can selectively bio-accumulate heavy metals particularly zinc and lead (Khedr and Hegazy, 1998 and Haroon and Daboor, 2009). It is a widely distributed species recorded in Romania, Egypt, Tropical Africa and Asia (Boulos, 1999). In Egypt, it was encountered in irrigation and drainage canals of the

Nile Delta. In southern Egypt, *N. lotus* is uncommon (El- Hadidi, 1971). In addition, the two species are not recorded in Nile valley and Nile Fayum (Tackholm, 1974: Boulos, 1995 and Zahran and Willis, 2003).

Since aquatic weeds are known to differ widely in their chemical composition depending upon species, season and location (Anon, 1984 and Haroon, 2008) an insight into their chemical composition is essential if utilization prospects are to be considered. The present study aims at evaluating the nutritive status of the two dominant and widely distributed hydrophytes along the water courses in Nile Delta, Egypt (*Pistia stratiotes* L. and *Nymphaea lotus* L.) in terms of estimating their organic and inorganic chemical compositions. In addition, the work involved screening for the presence of some anti-nutritive factors, the presence of which could limit utilization prospects. Such study may assist in understanding the importance of these plants in fishes and animal nutrition in Egypt.

2. Materials and Methods

2.1. Collection and drying of plant materials

Samples of aquatic weeds, *Pistia stratiotes* and *Nymphaea lotus* were collected in August, 2008 from irrigation canal lies at El-Serw Village, which situated at North Eastern part of the Nile Delta South of Manzalah Lake. Samples of *Pistia stratiotes* and *Nymphaea lotus* were collected from more than 10 individual plants within a 2 m stretch of the canal, and then they were mixed up to form a composite plant sample. Three plant samples were prepared for each species. The harvested plants were placed in polyethylene bags and transported to the laboratory where they were cut to include only shoots. These were then washed and additional moisture drained before being weighed and dried in shade. This is followed by another drying in an oven at 100 ºC to constant weight for dry matter determinations. A part of each plant sample was dried separately at 50 ºC to constant weight for estimation of biochemical constituents. All samples were ground to fine powder and stored until analyses could be done.

2.2. Proximate analysis

Humidity was considered as the loss in mass from drying sample at room temperature and drying at 100° C. Nitrogen (N), ash, ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE) were estimated using standard methods of the Association of Official Analytical Chemists (AOAC, 1990). Crude protein (CP) was calculated by multiplying the insoluble nitrogen by the factor of 6.25 (Ölberg, 1956). Nitrogen free extractives (NFE) comprising the sugars, starches and a large part of the material classed as hemi-cellulose was determined as the weight difference using crude protein, lipid, crude fiber and ash content data (McDonald, *et al*., 1973). Ash content was estimated by ignition at 500 ºC for about 24 hours. The crude lipid (EE) was extracted using a Soxhlet apparatus and quantity of lipid was determined gravimetrically. The crude fiber contents were estimated according to the Egyptian Pharmacopoeia (1953).

2.3. Calculated parameters

Digestible crude protein (DCP) was calculated according to the equation of Demarquilly and Weiss (1970): DCP (in % DM) = 0.929 CP (in %DM) – 3.52. Protein to energy ratio (P/E) was calculated as mg crude protein / K cal GE

The nitrogen free extracts (NFE) were calculated according to Pádua *et al*. (2004):
NFE (in %DM) = 100 – (humidity +CP +EE +CF +Ash); where CP = Crude Protein, EE = Ether Extract (total lipids) and CF = Crude Fiber.

Metabolized Energy (ME) was calculated according to Pantha (1982), using the values of 3.4, 8.1 and 4.2 Kcal 100 g^{-1} for carbohydrate, fat and protein respectively; ME (Kcal 100 g^{-1}) = 3.4 NFE + 8.1 EE +4.2 CP.

Gross energy (GE) was calculated following this equation (NRC, 1984):
GE (Kcal 100 g^{-1}) =5.72 CP +9.5 EE +4.79 CF +4.03 NFE. The results were expressed in Kcal 100 $g^{-1}$of DW, where, cal = Calorie, DW= dry weight. The results were then multiplied by 4.18/100 to be converted from Kcal 100 $g^{-1}$of DW to K J g^{-1} of DW, where, J= Joule, DW= dry weight.

2.4. Mineral analysis

For mineral analyses ash samples were dissolved in nitric acid (HNO_3, 50% v/v) and diluted with double distilled water (1:10). The mineral elements of the plants, namely; Na and K were estimated by flame photometry, whereas Fe, Zn, Cu and Mn contents were measured by atomic absorption spectrophotometry (Carl Zeiss) using standard reference chemicals (Allen *et al.* 1986). The total phosphorus content was determined as described by Umoren *et al*. (2005).

2.5. Amino acids analysis

The powdered samples of the selected species was subjected to investigation of amino acids composition using Automatic Amino Acid Analyzer as described below. Acid hydrolysis was carried out according to the method of Block *et al*. (1958). AAA 400 INGOS Ltd Automatic Amino Acid Analyzer was used for this purpose. Standard samples were also injected under the same conditions of the investigated samples. Retention times and peaks areas were determined using Hewlett-Packard 3390 recording integrator. Peak identification was performed by comparing the relative retention time (RRT) of each amino acid detected in sample with those obtained for standards. The concentration of each

amino acid (GM/ 16GM nitrogen) was calculated using a specially designed program.

2.6. Fatty acids analysis

The identification and quantitative determination of fatty acids in the plant powders were carried out using Gas Liquid Chromatography Trace GLC Ultra. The method of AOAC (2000) was conducted for lipid extraction from samples using chloroform methanol (2:1 v/v), while separation and methylation of fatty acids were carried out following the method of Vogel (1975), followed by samples injection into the gas chromatography. A set of standard fatty acids of 10:0, 12:0,13:0, 14: 0, 15:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1 and 22:0 with a stated purity of 99% were also injected into GLC at the same condition of the samples and the relative retention times (RRT) were calculated. The method described by Farag *et al*. (1986) was used as a guide to characterize some of the unknown compounds.

2.7. Total phenolics and phytochemical screening

Folin–Ciocalteu method (Meda *et al*., 2005) was used to determine the total phenolic content of the plant extracts and the data were expressed as milligram gallic acid equivalents (GAE) / 100g dry matter from plant samples**.** The preliminary phytochemical screening was assessed following the methodology of (Harborne, 1998 and Kokate, 2001).

2.8. Statistical analysis

One-way ANOVA was applied to assess the significance of variations in elements, organic components, and nutritive variables in relation to plant species and part of plant used (SAS, 1996). Data analysis was done using multiple interval test (Duncan), and testing was done for the level of significance of $p< 0.05$.

3. Results

Humidity values varied between 7.1 % in *N. lotus* stem and 8.20 % in *P. stratiotes* shoots ($P< 0.05$ between different species). Among the studied samples *P. stratiotes* shoots were shown to possess the highest amounts of ash (18.2 %), whereas the highest organic matter content (86.6 %) was recorded in *N. lotus* leaves (Table 1). All samples contained high protein contents with a maximum value 19.5 % in *N. lotus* stem. Lipid contents ranged from 2.3 to 3.5 % in *N. lotus* leaves and *P. stratiotes* respectively. The highest crude fiber contents 26.3 % was found in *N. lotus* stem, while the highest NFE 35.1 % in *N. lotus* leaves. In addition, the highest value of digestible crude protein (DCP 14.6 %), growth energy (GE 16.0 K J g^{-1} of DW), the ratio of protein to energy (12.2), protein to lipids (7.8) and protein to nitrogen free extracted ratio (0.6 %) were recorded for *N. lotus* stem.

N. lotus stem had the highest concentrations of N (3.1 %) and Na (1.0 %); while *P. stratiotes* had the highest concentrations of P (0.4 %) and *N. lotus* leaves had the highest concentrations of K (3.3 %). On the other hand, *P. stratiotes* shoots had the ability to accumulate more concentrations of Fe, Mn , Zn and Cu than the other species (Table 2).

The total determined amino acids (Table 3) were higher in *N. lotus* leaves than that of *N. lotus* stem and *P. stratiotes* shoots (76.6, 72.2 and 67.1 % of the total protein respectively). Seventeen of the common amino acids were recorded in each species, varied in their concentrations with plant species and a part of plant used. Glycine and aspartic acid represent the major constituent of non essential amino acids separated, while leucine and valine were the major representative essential amino acids. In addition, the two sulpher containing amino acids methionine and cystine were not detected.

The results of fatty acids analysis revealed the presence of different numbers and concentrations which varied with plant species and a part of plant used (Tables, 4, 5 and 6). *P. stratiotes* shoot and *N. lotus* stem samples were characterized by the presence of capric acid, which was absent in *N. lotus* leaves. Moreover, myristic acid (saturated fatty acid) represents the highest amount of fatty acids separated from *P. stratiotes* shoots (28.3 %) and *N. lotus* leaves (24.0 %) extracts, while linolenic acid (unsaturated fatty acid) represent the major constituent of *N. lotus* stem (34.0%) fatty acids. Comparing the two studied species (Table 7) *N. lotus* stem had the highest value of total unsaturated fatty acids (54.8 %), while *P. stratiotes* had the highest value of total saturated fatty acids (63.5 %).

Phenolic contents of the studied plants varied between (2.7%) in *N. lotus* stem and (2.4%) in *P. stratiotes* shoots ($P< 0.05$ between different species and different parts of the same species). The preliminary phytochemical screening have revealed the presence of flavonoids, alkaloids, tannins, steroids, chlorides and sulphates in the studied plant extracts (Table 8). However, resins were not detected in *P. stratiotes* shoots and saponins were detected only in *N. lotus* leaves extract.

Table 1. Mean ± SE of the nutritive values of *N. lotus* and *P. stratiotes* (% of DW), values with the same letters ([a]- [c]) within the row are not significantly different at (P < 0.05). Digestible crude protein (DCP), Growth energy (GE), Metabolized energy (ME).

Plants / Parameters	*P. stratiotes* shoots	*N. lotus* leaves	*N. lotus* stem
Humidity.	8.2 [a] ± 0.1	7.3 [b] ± 0.12	7.1 [b] ± 0.0
Organic Matter	81.8 c± 0.0	86.6 a ± 0.04	85.7 b ± 0.1
Ash	18.2 [a] ± 0.0	13.4 [c]± 0.04	14.3 [b]± 0.1
Protein	15.2 [c] ± 0.0	16.5 [b] ± 0.02	19.5[a] ± 0.0
Lipid	3.5 [a] ± 0.0	2.3 [c] ± 0.03	2.5[b] ± 0.0
Crude Fiber	22.1 [c] ± 0.3	25.5 [b] ± 0.03	26.3 [a] ±0.1
NFE	32.9[b] ± 0.2	35.1 [a]± 0.17	30.4 [c] ± 0.1
DCP (%)	10.6 [c] ± 0.19	11.8 [b] ± 0.2	14.6 [a] ± 0.2
GE (K J g^{-1})	15.0 [c] ± 0.75	15.8 [b] ± 0.7	16.0 [a] ±0.3
ME (K J g^{-1})	8.5 [b] ± 0.63	8.6 [a] ±0.8	8.6 [a] ± 0.5
P/E	10.1 [c] ± 0.01	10.4 [b] ± 0.0	12.2 [a] ± 0.1
P/L	4.4 [c] ± 0.05	7.3 [b] ± 0.1	7.8 [a] ± 0.1
P/NFE	0.5 [b] ± 0.00	0.5 [c] ±0.0	0.6 [a] ± 0.

Table 2. Mean values ±SE of macro and micro elements in *P. stratiotes* and *N. lotus* shoots, values with the small different letters ([a]- [c]) within the row are significantly different at (P < 0.05).

Plants	*P. stratiotes*	*N. lotus* leaves	*N. lotus* stem
Macro elements (% of DW)			
N	2.4 [c] ± 0.0	2.6 [b] ± 0.0	3.1 [a] ± 0.0
P	0.4 [a] ± 0.0	0.3 [b] ± 0.0	0.3 [b] ± 0.0
K	3.1 [b] ± 0.0	3.3 [a] ± 0.0	2.9 [c] ± 0.01
Na	0.5 [c] ± 0.0	0.5 [b] ± 0.0	1.0 [a] ± 0.0
Micro elements (µ g g^{-1} of DW)			
Fe	4480.4 [a] ± 0.1	739.2 [c] ± 0.0	2178.7 [b] ± 0.0
Mn	894.0 [a] ± 0.1	165.1 [c] ± 0.0	578.2 [b] ± 0.0
Zn	240.7 [a] ± 0.3	187.4 [c] ± 0.0	200.5 [b] ± 0.0
Cu	20.1 [a] ± 0.0	4.1 [c] ± 0.1	19.3 [b] ± 0.1

Table 3. Essential and non essential amino acids yield of *P. stratiotes* and *N. lotus* shoots proteins (% of crude protein**).**

Amino acids	*P. stratiotes* (shoots)	*N. lotus* (leaves)	*N. lotus* (stem)
Aspartic acid.	10.7	9.7	9.6
Threonine *	3.9	3.9	2.8
Serine.	4.5	5.0	4.7
Glutamic acid.	7.9	9.3	8.3
Proline	0.1	0.1	0.1
Glycine.	11.9	11.0	11.2
Alanine.	9.5	9.0	9.6
Cysteine.	-		-
Valine *	4.9	5.0	4.2
Methionine.	-	-	-
Lsoleucine. *	2.5	2.7	2.3
Leucine. *	5.6	6.4	3.8
Tyrosine *	1.7	3.1	1.0
Phenyl alanine. *	1.0	1.8	2.6
Histidine. *	1.1	1.9	1.2
Lysine. *	4.5	4.4	3.6
Arginine *	2.5	3.2	2.4
Total amino acids	72.2	76.6	67.1
Total essential *	27.6	32.5	23. 7
Total nonessential	44.6	44.1	43.5
Ammonia.	27.8	23.4	32.9

Table 4. Fatty acids of *P. stratiotes* shoots separated by Gas Liquid Chromatography (GLC).

Peak no.	Area %	Carbon no.	Chemical name	Common name
1	1.4	C 8:0	Octanoic acid	Caprylic
2	1.4	C 10:0	Decanoic acid	Capric
3	8.1	C 12:0	Dodecanoic acid	Lauric
4	28.3	C 14:0	Tetradecanoic acid	Myristic
5	13.7	C 14:1	Cis- 9- Tetradecanoic	Myristoleic
6	16. 9	C 16:0	Hexadecanoic	Palmitic
7	6.5	C18:0	Octanoic acid	Stearic
8	8.6	C 18:1	Cis-9- Octadecenoic	Oleic
9	6.3	C 18:2	Cis, Cis-9,12- Octadecadienoic	Linoleic
10	5.6	C 18:2	Cis, Cis-9,12- Octadecadienoic	Linoleic
11	1.1	C 18:;3	All cis-9,12,15- Octadecatrienoic	Linolenic
12	1.2	C 20:0	Eicosanoic acid	Arachidic
13	0.9	unknown	-	-

Table 5. Fatty acids of *N. lotus* leaves separated by Gas Liquid Chromatography.

Peak no.	Area %	Carbon no.	Chemical name	Common name
1	4.3	unknown	-	-
2	4.0	unknown	-	-
3	11.9	unknown	-	-
4	1.0	C 8:0	Octanoic acid	Caprylic
5	7.0	C 12:0	Dodecanoic acid	Lauric
6	24.0	C 14:0	Tetradecanoic acid	Myristic
7	11.9	C 14:1	Cis- 9- Tetradecanoic	Myristoleic
8	15.5	C 16:0	Hexadecanoic	Palmitic
9	4.3	C18:0	Octanoic acid	Stearic
10	4.7	C 18:1	Cis-9- Octadecenoic	Oleic
11	5.9	C 18:2	Cis, Cis-9,12- Octadecadienoic	Linoleic
12	3.0	C 18:2	Cis, Cis-9,12- Octadecadienoic	Linoleic
13	2.2	C 18:;3	All cis-9,12,15- Octadecatrienoic	Linolenic
14	0.5	C 20:0	Eicosanoic acid	Arachidic

Table 6. Fatty acids of *N. lotus* stem separated by Gas Liquid Chromatography.

Peak no.	Area %	Carbon no.	Chemical name	Common name
1	1.1	C 8:0	Octanoic acid	Caprylic
2	1.2	C 10:0	Decanoic acid	Capric
3	7.7	C 12:0	Dodecanoic acid	Lauric
4	1.4	Unknown	-	-
5	25.0	C 14:0	Tetradecanoic acid	Myristic
6	13.7	C 14:1	Cis- 9- Tetradecanoic	Myristoleic
7	7.1	C 16:0	Hexadecanoic	Palmitic
8	6.3	C 18:1	Cis-9- Octadecenoic	Oleic
9	0.9	C 18:2	Cis, Cis-9,12- Octadecadienoic	Linoleic
10	34.0	C 18:;3	All cis-9,12,15- Octadecatrienoic	Linolenic
11	1.8	C 20:0	Eicosanoic acid	Arachidic

Table 7. Comparative total saturated, unsaturated and saturated to unsaturated fatty acids of *P. stratiotes* and *N. lotus* shoots. TSFA= Total saturated fatty acids, TUSFA= total unsaturated fatty acids and Sa/Unsa. = The ratio of saturated to unsaturated fatty acids.

Plants	*P. stratiotes* shoots	*N. lotus* leaves	*N. lotus* stems
TSFA	63.5	52.2	43.8
TUSFA	35.4	27.7	54.8
Sa/Unsa	1.8	1.9	0.8

Table 8. Total phenolic contents (means ± standard error) and phytochemical screening of *N. lotus* and *P. stratiotes* shoots extracts. Values with the same letters within the row are not significantly different at ($P < 0.05$). + Present at small concentrations, ++ Present at moderate concentrations, +++ Present at high concentrations.

Samples	*P. stratiotes*	*N. lotus* leaves	*N. lotus* stem
Total phenols (g/100g)	$2.5^{b} \pm 0.0$	$2.4^{c} \pm 0.0$	$2.7^{a} \pm 0.0$
Bioactive agents			
Flavonoides	++ve	++ve	++ve
Alkaloides	+ve	+ve	+ve
Tannins	++ve	++ve	+++ve
Sterols	+ve	+ve	+ve
Resins	- ve	+ve	+ve
Saponins	- ve	+ve	-ve
Chlorides	+ve	+ve	++ve
Sulphates	+ve	+ve	++ve

4. Discussion

Measurable differences in nutritional composition were apparent among the species studied. Six major components were considered when analyzing the selected plants as a potential animal and fish feed: (energy, carbohydrates, crude protein, crude fat, macro-elements and micro-elements). Energy is essential for the maintenance of life processes including, cellular metabolism, growth, reproduction and physical activity. So, the ability of a food to supply energy is of a great importance in determining its nutritional value to animals (Bligh and Dyer, 1959). In the present investigation *N. lotus* and *P. stratiotes* showed a significant difference in gross energy, but exhibited remarkable similarity in possessing nearly the same metabolized energy. Carbohydrates include sugars, starches, cellulose and other related compounds and serve as the principle source of metabolizable energy in terrestrial farm animals. In fish and shellfish, no essential dietary requirement for carbohydrates has been established. Carbohydrates are included in fish and shellfish diets because they are an inexpensive source of dietary energy, serve as a binding agent during feed manufacturing and can increase feed palatability (Craig and Helfrich, 2002 and Bureau and Cho, 2003).

Carnivorous fish species (salmonids) have a limited ability to digest complex carbohydrates due to the weak amylotic activity in their digestive tract. By contrast, warm water omnivorous and herbivorous fish species such as carp, channel catfish, tilapia and eel have been found to be more tolerant to high dietary carbohydrate levels. Unlike terrestrial farm animals, most fish species have a relatively short gastro intestinal tract with little microbial colonization. As a result, the intestinal cellulase activity of fish is weak or absent. Consequently, dietary cellulose or crude fiber has no utilizable energy value and in dietary excess has a negative impact on growth and feed efficiency (FAO, 1987).

Carbohydrate contents ranged from 30.4 to 35.1 % and crude fiber contents ranged from 22.1 to 26.3%. These not exceed the dietary requirements of fish and shellfish for carbohydrates 10- 30%, but exceed that for crude fibers 1.1 % (Barrias and Oliva – Teles, 2000 and Royes and Chapman, 2003). At the same time, these values lay with the range of some rough fodder material 27.8–51.9 % (Shoukry, 1992), but lower than those of *Trifolium alexandrinum* 43.4 % (Chauhan *et al*., 1980), *Echinochloa stagnina* and, *Eichhornea crassipes* 53.7 and 54.1% (Shaltout *et al*., 2010**)** and *Eichhornea crassipes and Echinochloa stagnina* leaves 38.9 and 45.7% (Sharshar and Haroon, 2009). In addition, the crude fibers content in *N. lotus* samples were higher than that of *T. alexandrinum* 21.5 % (Chauhan, *et al*., 1980), while those of *P. stratiotes* was relatively similar. Moreover, the emergent *N. lotus* plant had higher crude fiber content compared with the free floating plant *P. stratiotes*, this probably because emergent species require more strength to support aerial vegetation (Banerjee and Matai, 1990).

Proteins have crucial functions in all the biological processes. Their activities can be described by enzymatic catalysis, transport and storage, mechanical sustentation, growth and cellular differentiation control (S¨ukran, *et al*., 2003). Protein requirements vary depending on species cultured, rearing environment and size and age of the cultured organisms. Generally, herbivorous fish have lower protein requirements than omnivorous and carnivorous species, fish reared in low density systems (pond aquaculture) have lower protein requirements than fish reared in high density systems (recirculating aquaculture) and larger, older fish have lower protein requirements than younger, smaller fish (Wilson, 2002).

Ministry of Agriculture, Fisheries and Food in England (Anonymous, 1975) reported that minimum protein in the animal diet ranges between 6 to 12% depending on the animal species. The average protein contents in the studied samples ranged from (15.2 to 19.5 %). These do not meet the dietary requirements of aquatic animals 32- 52% reported by (Barrias and Oliva – Teles, 2000 and Royes and Chapman, 2003), but higher than the proper level reported for animal diet

(Anonymous, 1975). In addition, the samples under investigation were higher in their protein contents compared with the other fodder plants such as *Trifolium alexandrinum* 16.2%: (Chauhan *et al*., 1980), hydrophytes *Echinochloa stagnina, Eichhornia crassipes* and *Ceratophyllum demersum* (Shaltout *et al*., 2010). *Echinochloa stagnina, Eichhornia crassipes* and *Potamogeton tomentosum* (Sharshar and Haroon, 2009). At the same time, the results are not comparable to those reported for the same species by Abu Ziada *et al*. (2008) and Snow and Ghaly (2008) this may be related to the part of plant used, plant growth form, time and place of samples collection.

The recovery of amino acids from acid hydrolysis accounted 72.2, 76.6 and 67.2 % of total proteins for *P. stratiotes* shoots, *N. lotus* leaves and *N. lotus* stem respectively; indicating either incomplete hydrolysis or destruction of some amino acid. However, the amount of essential amino acids in plants under investigation can be compared to the F.A.O. reference pattern (Burton, 1965) it showed deficient levels in isoleucine. In addition, *P. stratiotes* shoots and *N. lotus* leaves showed a deficient level in phenylalanine, while *P. stratiotes* and *N. lotus* stem showed a deficient level in tryosine. Lysine and tryptophan are the first two limiting amino acids in grains, therefore interest centers on the concentration of these two amino acids in plants under investigation. A diet containing an adequate protein level (1g/ kg body weight) will be balanced in lysine if it contains 4.2 g of that amino acid/ 100 g of protein (Taylor and Robbins, 1968). *P. stratiotes* and *N. lotus* leaves containing from 4.5 to 4.4% respectively, however Corn is deficient in lysine, containing only 0.8% (Block *et al.*, 1958), which meaning the possibility of using these plants to improve lysine content of a corn diet. Moreover, if subsequent work on tryptophan content of plants under investigation reveals the occurrence of that amino acid in substantial amounts, the proteins of these plants may be developed into useful dietary supplements for grain diets, especially in the underdeveloped countries.

Determination of protein to energy ratio (P/E) in fish diet is very important because the higher this ratio, the better is the diet (Snow and Ghaly, 2008). Therefore, on the basis of high gross energy, as well as P/E values, it may be inferred that *N. lotus* stem and leaves are suitable for incorporation in fish diet to reduce the cost of fish feed.

Lipids are required for the long-term storage of metabolic energy, to supply essential fatty acids, as carriers of fat soluble vitamins and for structure and control (FAO, 1987). However, lipids of *N. lotus* and *P. stratiotes* (2.3 - 3.5%) do not meet the dietary fat requirement of fish and shellfish (4-28%: Snow and Ghaly, 2008), it lies within the scale of some rough fodder materials (0.5%–3.1%: (Shoukry, 1992) and being, higher than that of *T. alexandrinum* (2.9%) (Chauhan *et al*., 1980).

In fish fatty acids are the major source of metabolic energy needs, for growth, reproduction and egg production (Sargent, *et al*., 2002). In the present investigation both omega -3, and 6 fatty acids were detected in the two species, but with different concentration varied with plant species and part. *N. lotus* stem had the highest concentration of total unsaturated fatty acids 54.8 %, while *P. stratiotes* had the highest concentration of total saturated fatty acids 63.5, which reduced its nutritional value as compared with *N. lotus* samples.

Although adequate levels of essential mineral nutrients are the important aspect of nutritive quality, excessive concentration of ash decreases the amount of organic constituents per unit weight and lowers food value (Polisini and Boyed, 1972). In some green roughages commonly used as livestock feed, ash content ranged from 8.6% in maize to 14.2% in cowpea (Banjeree, 1988). In the present investigation ash, value of *N. lotus* lies in this range, which meaning the suitability of this species to be used as animal feed. Macroelements are required by the body in relatively large amounts (> 100 mg kg-1 dry diet), while microelements are required by the body in trace amounts (< 100 mg kg-1 dry die) (Snow and Ghaly, 2008) These elements function in cellular metabolism, have important roles in osmoregulation and acid-base balance and serve as structural components of tissues (Jobling, 2001). The studied plants meet the P, Cu, and Zn dietary requirements of aquatic animals and exceed the K, Na, Fe and Mn requirements.

Phenolic contents of the plants were studied in view of the adverse effect of these compounds on growth due to their interference with protein digestibility (Bondi and Alumot, 1987). Plants having a tannin content of 6% and above have been reported to be low in digestibility (McLeod, 1974) and hence of little feed value. The anti-nutritional substances represented by total phenolic contents in the studied species lies in the suitable range (2.7 - 2.4%) for digestibility. These results are comparable with those previously reported by (Anjana and Matai, 1990). In addition, the preliminary phytochemical screening of the three plants extracted suggested the presence of some biologically active substances which, have been recorded for their therapeutic effects. At the same time, the antimicrobial activity of the two species was recorded by (Abu Ziada, *et al*., 2008), while Yisa (2009) and Akinjogunla, *et al*. (2009) recorded it for *N. lotus*. In conclusion the studied plants were found to contain sufficient quantities of nutrients and were safe enough to be considered as potential animals feed, but it did not contain sufficient amounts of protein and fat to meet the dietary requirements of fish and shellfish. In addition, these plants contain sufficient amount of lysine and could serve to improve the lysine content of some grain diets, moreover, the two species were characterized by the presence of omega 3 and 6 fatty acids and could be important sources of amino acids, fatty acids and minerals, suitable for incorporation in fish diet. In general, this study reported that, *N. lotus* stem has high protein, GE, P/E, P/L, P/NFE and

unsaturated fatty acid contents, thus it may be the most preferable feeding item for animals and fishes.

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تقييم الحالة الغذائية للمجموع الخضرى لنباتى البشنين الأبيض و الزقيم بغرض إستخدامهما كغذاء للأسماك و الحيوان

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أجريت هذه الدراسة بهدف البحث عن مصدر غذائى جديد يكون مناسب لخفض تكلفة الغذاء الصناعى و ذلك من خلال تقييم الحالة الغذائية لنوعين من النباتات المائية المعروفة و المنتشرة بوفرة فى دلتا النيل و هما نبات البشنين الأبيض و نبات الزقيم اللتان تم تجميعهما من إحدى قنوات الرى بمدينة السرو جنوب بحيرة المنزلة فى الشمال الشرقى لدلتا النيل. و قد تناولت الدراسة تعين المركبات العضوية و غير العضوية فى النباتات محل الدراسة بالإضافة إلى تقدير الأحماض الأمينية و الدهنية كماً و نوعاً, هذا بالإضافة إلى عمل تقدير كمى للمركبات الفينولية و عمل مسح كميائى لبعض المركبات الهامة طبياً داخل النباتات . ومن نتائج الدراسة تبين أن ساق نبات اليشنين الأبيض قد تميز بإحتوائه على أعلى نسبة من البروتين الخام و المهضوم و الطاقة اللازمة للنمو و أعلى نسبة من البروتين بالنسبة للطاقة و الدهون و الكربوهيدرات. كما دل تحليل البروتين على و جود عدد 15 نوع من الأحماض الأمينية الأساسية و غير الأساسية داخل كل نبات و لكنها إختلفت كماً و نوعاً تأثراً بنوع النبات و أجزاء النبات الواحد. كما أسفر التحليل الكروماتوجرافى للأحماض الدهنية بإستخدام جهار GLC عن و جود إختلاف فى أنواع و نسب هذه الأحماض الدهنية تأثراً بنوع النبات و أجزاؤه, حيث تميزت عينات ساق نبات البشنين الأبيض بإحتوائها على أعلى نسبة من من حمض لينولينك (أوميجا 3) بينما تميزت أوراق نفس النبات و نبات الزقيم بوجود تركيز عالى من حمض اللينوليك (أوميجا 6) . كما سجل تحليل العناصر الصغرى و الكبرى و جود إختلاف واضح فى تركيزها تبعاً لنوع النبات و الجزء المستخدم منه. و قد تراوح التركيز الكلى للمركبات الفينولية بين 2.4- 2.7% و دل المسح الكميائى لمستخلصات هذه النباتات على و جود بعض المواد ذات الأهمية فى الطب العلاجى مثل الفلافونيدات و القلوينيدات و الدباغيات و الإسترويدات و الكلوريدات و الكبريتات فى كل العينات.

و من نتائج هذه الدراسة يمكن الإشادة بالقيمة الإقتصادية لهذه النباتات كمصدر غذائى جديد و متوازن يمكن الإستفاده منه فى تقليل تكلفة الغذاء الصناعى إذا ما تم خلط هذه النباتات و على الأخص نبات البشنين الأبيض بجزئيه مع الغذاء الصناعى لكلاً من الأسماك و الحيوان.