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Phytochemical Studies on Mangrove and the Possibility of Using it as Fodder

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ABSTRACT. Chemical composition of two mangrove species (*Avicennia marina* and *Rhizophora mucronata*) for leaves, stem and root were studied including proteins, amino acids, fatty acids and metals. The objectives is to examine the possibility of using the plant as part of the conventional feed used in fish or other farms. The study also describes fish *(T. aurea)* nutrition experiments using mangrove leaves as part of the diet. The dietary composition was made in view of the results of the chemical and calorific analysis of plant parts. Results of analysis have, in general, pointed out to relatively higher contents of protein, carbohydrates, and lipids in the mangrove leaves compared to other parts of the plant. Although the experiments gave nutritionally negative results (as expected) with respect to mangrove-containing diet as compared to the control (commercial) diet, there were no indications of biologically adverse effects on the feeder. It has been suggested that the reason for this result may be attributed to tough cell walls of the mangrove leaves, or to the presence of enzymatic and metabolic inhibitors within the leaves.

Introduction

The idea of using components of mangrove plants as animal fodder is not a new one and their feasibility to be used as such had been demonstrated in a number of fairly dated studies, Sokoloff *et al.* (1950), Khar and Neji (1953) and Khar (1954). However, with the ever rising cost of fodder due to global shortages in traditional food resources, the idea has revived again, and many studies dealing with this issue have been published relatively recently; *e.g.* Waghmode and Joshi (1982), Mirza and Cresser (1985), Qasim *et al.* (1986) and Khafaji *et al.* (1988).

man *et al.* 1957) and quantified spectrophotometrically using pure amino acid standards, on a Unicam sp 1800 spectrophotometer. For tryptophan, hydrolysis was made using barium hydroxide and quantified according to the method of Blauth *et al.* (1963). After conversion to the methyl esters using diazomethane (Vogel, 1975), fatty acids were analyzed by gas chromatography on a GCU Pye Unicam gas chromatograph. Chromatographic conditions were as follows: Column packed with PEG adipate, supported on silanized diatomite C (100-120 mesh) with isothermal column temperature held at 190°C, FID temp. at 200°C and nitrogen flow rate of 30 ml/min.

After acid digestion, sodium and potassium concentrations were determined flame photometrically, whereas phosphorus was determined spectrophotometrically as phosphomolybdic acid. The rest of the elements were determined by atomic absorption spectroscopy on a Unicam SP 1900 AA spectrophotometer. Calorific values were obtained by direct measurements using a Parr 1241 adiabatic bomb calorimeter.

Biological experiments were carried out on the fish species, *Tilabia aurea.* Five treatments of five different feed compositions were tested. In one treatment, the control experiment, a pure commercial fish diet was used. This has been marketed as containing a mixture of unspecified fractions of sorghum, soya beans, fish, bones and meat powders. The rest of the treatments contained mangrove powdered leaves added as supplements to the above mentioned commercial diet according to the following percentage: 40% *Rhizophora,* 40% *Avicennia,* 80% *Rhizophora* and 80% *Avicennia.* All of the five types of feed were supplied to the feeders in the form of pellets. This was done by milling the dried food into powder, wetting it with water to form a paste and finally pelletizing it into small pellets of about 2.5mm in diameter. The pellets were then air dried for 2 days, dried with hot air in a special drying chamber for 3 hours and finally stored in the cold for future use.

Food mixing was carried out on dry weight basis, and daily diets were determined as a constant percentage of the total fish weight per tank. To feed the fish on dry weight basis, the moisture content of each of the five types of feed was determined separately as mentioned before.

Nutrition experiments were carried out in an open system under flowing conditions. Circular fiber glass tanks of 72 cm diameter and 50 cm depth, were used in the experiments. Triplicates of 20 individual fishes per tank, weighing 4.3-5.0 g each, were acclimated over a week's time feeding only on commercial diet. Each tank was equipped with a central draining pipe, concentric with another larger pipe fitted at the bottom to allow drainage of the tank from below. The flowing sea water was adjusted to run at a flow rate of 2.5 to 3 liters per minute in each tank. After acclimation, fishes were reweighed and redistributed in the tanks. A diet equivalent to a dry weight of feed, amounting to 4% of the total fish weight per tank, was supplied in 3 rations given daily at 8:00 AM, noon, and 3:00 PM over the first week. The total diet was increased to 6% as of the second week. Growth was monitored by weighing the fishes weekly and readjusting the necessary amount of the daily food supply.

(1986) who studied a monospecific stand of the same species. This latter agreement in the protein values of *A. marina* from different localities is probably indicative of similarity in environmental conditions in relation to requirements for protein synthesis by the plant.

Protein Content

Initial attempts for extraction of proteins using the conventional methods were rather unsuccessful. The lack of success was apparently due to an unusual toughness in the plant cell walls. For this reason, the extraction was carried out according to a method used for algal proteins, by Young (1970) with some modification introduced by Shallan (1984). The difficulty of protein extraction and the presumed toughness of the cell provided an early alarm of the possible cell wall hindrance of protein assimilation by prospective feeders, and hence a possible deleterious effect on their growth.

Electrophoretic analysis of proteins gave seven separate groups for each of the two species, *Rhizophora* and *Avicennia* (Fig. 1 and 2). Four of these groups, labelled 2, 3, 5 and 6, precipitated on treatment with ammonium sulfate of concentrations up to 50% saturation, indicating that they are different types of globulins. The rest of the protein groups, 1, 4 and 7, precipitated at concentrations of ammonium sulfate ranging from 50 to 100% saturation, indicating that they are different types of albuminoid proteins (Fig. 1 and 2).

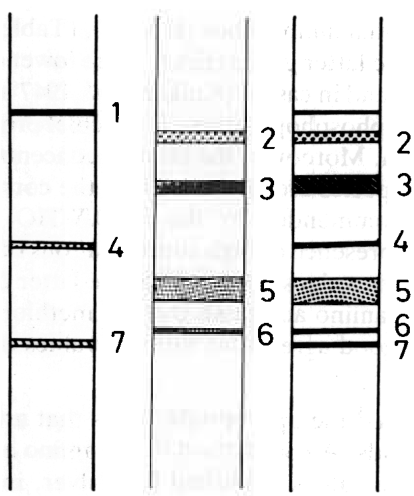

FIG. 1. a. Protein fraction precipitated with 50-100% Satn. Ammon. Sulphate.
b. Protein fraction precipitated with 1-50% Satn. Ammon. Sulphate.
c. Electrophoretic pattern of extracted Rhizophora leaf proteins.

TABLE 2. Percent amino acid in the leaves of mangrove species (± 1 S.D.

% Amino acid composition of proteins		
Amino acids	*Avicennia marina*	*Rhizophora mucronata*
Glycine	6.63 ± 0.21	5.29 ± 0.34
Cystine	17.92 ± 0.16	18.51 ± 0.21
Methionine	4.16 ± 0.11	3.83 ± 0.19
Leucine	2.19 ± 0.06	1.82 ± 0.09
Leucine + Isoleucine	5.95 ± 0.13	4.39 ± 0.07
Phenylalanine	1.94 ± 0.19	1.02 ± 0.08
Valine	1.52 ± 0.11	1.21 ± 0.04
Threonine	2.08 ± 0.11	1.89 ± 0.12
Histidine	1.21 ± 0.09	1.95 ± 0.07
Arginine	2.63 ± 0.14	2.36 ± 0.18
Tyrosine	4.20 ± 0.09	5.04 ± 0.12
Alanine	2.15 ± 0.12	2.63 ± 0.13
Aspartic	15.81 ± 0.25	17.37 ± 0.21
Glutamic	4.45 ± 0.07	5.14 ± 0.20
Proline	1.24 ± 0.06	1.93 ± 0.17
Serine	2.19 ± 0.21	1.53 ± 0.15
Tryptophan	0.95 ± 0.08	0.73 ± 0.06

TABLE 3. % Essential amino acids in the leaves of mangrove species and in casein together with the FAO/WHO recommended values.

% Essential amino acid in total proteins				
Amino acids	*Avicenia marina*	*Rhizophora mucronata*	Casein[(a)]	FAO/WHO Repast
Arginine	2.63	2.36	6.6	–
Histidine	1.21	1.95	2.4	–
Leucine + Isoleucine	5.95	4.39	14.8	7
Lysine	2.19	1.82	7.5	5.5
Methionine[(b)]	4.16	3.83	3.3	3.5
Phenylalanine[(c)]	1.94	1.02	4.8	6
Threonine	2.08	1.89	4.7	4
Tryptophan	0.95	0.73	1.5	1
Valine	1.52	1.21	6.8	5

(a) Cole (1950) Tryptophan value from Kuiken *et al.* (1947).
(b) Methionine + cystine.
(c) Phenylalanine + tyrosine.

saturated fatty acids was greater than the total saturated acid content. The ratios of the total unsaturated to the total saturated fatty acids were 1.31 and 1.44 for *Rhizophora* and *Avicennia* respectively. The highest concentration was recorded for the saturated fatty acid, palmitic (16:0). Its value for *Rhizophora* and *Avicennia* respectively, amounted to 28.17 and 26.33% of the total fatty acid content. In addition, the analysis also revealed the presence of other common fatty acids characteristic of plant tissues, *e.g.* meristic (14:0), stearic (18:0) and oleic (18:1). Moreover, the per cent compositions of two of the unsaturated fatty acids that are believed to be of nut-

TABLE 5. Element contents of mangrove species.

Element	*Avicennia marina*	*Rhizophora mucronata*
Sodium	2.98 %	1.81 %
Potassium	1.43 %	0.69 %
Calcium	1.63 %	1.26 %
Phosphorus	0.12 %	0.14 %
Magnesium	0.78 %	0.62 %
Iron	133 ppm	109 ppm
Manganese	102 ppm	216 ppm
Zinc	46 ppm	34 ppm
Copper	7.5 ppm	5.4 ppm

dation states, Mn III and Mn IV, it precipitates and assumes an insoluble or particulate form. For this reason, in anaerobic (anoxic) aquatic environments the soluble reduced forms, manganous, dominates over other forms. If oxygen is introduced, a chemical reaction takes place and the soluble form is oxidized to the insoluble form and precipitates. However, this reaction is a pH-dependent and is particularly favoured in alkaline media.

As it has already been mentioned, the *Avicennia,* by virtue of its close proximity to the sea receives more of the fresh oxygenated sea water than does the distal *Rhizophora.* This fresh influent water is apt to enrich oxygen in the water logged *Avicennia* swamp and to step up its oxidizing power. The net result will be an increase in the efficiency of manganese oxidation and precipitation reactions; with a concomitant decrease in the soluble fraction available to the plant.

An alternative, or may be cooperative, factor that may also help in explaining the lower manganese level in *Avicennia* leaves compared to *Rhizophora,* may be found in the different nature of the root system in each species. The *Avicennia* root system is of the pneumatophoric type of root which protrudes well above the ground to ensure adequate aeration of the plant at all times. On the other hand, the root system of *Rhizophora* is of the prop root type which penetrate much deeper in the soil. So, there appears to be a good chance that appreciable amounts of the soluble manganese which is absorbed by the surfacial *Avicennia* roots may become oxidized and deposited in the well aerated roots of the plant before it can be transported to the leaves. By contrast, the deeper penetrating *Rhizophora* roots are more likely to absorb most of their manganese budget from the deep anaerobic interstitial water where soluble manganese is plentiful and may be further enriched by mobilization from sediments to the aqueous interface. In fact, this latter explanation may appear more plausible in context of the description given by Mandura *et al.* (1987). They described the *Avicennia* species as to have profusely growing pneumatophores extending in height to about 15 cm above ground and reaching a density as high as 400 roots per square meter. On the other hand, Mandura *et al.* (1987), described a *Rhizophora* stand as to be growing in an extremely sheltered embayment almost closed except for a small opening to the outside. They also described the *Rhizophora* substratum as a soft muddy one, well suited for the viviparous species to germinate and for its prop roots to penetrate.

formity of the temperature of the aqueous medium has been shown to unify its effect on the growth rate of fish and also its effect on food digestion and assimilation (Talfan and Salman 1982). Another factor that is thought to affect the ability of fish to assimilate proteins is its size (or weight). Despite this belief, some studies (Talfan and Salman 1982) showed that various fishes of various sizes and age are equally able to assimilate proteins. However, to avoid this controversy, fishes used in the present investigation were selected within a narrow range of weight. Data presented in Table 6 indicate that both types of mangrove leaves do not contain lethally toxic substances, since fish mortality was nil in all cases. Further inspection of Table 6 indicates that at the end of the experiment, the control fish species, which were fed on pure commercial fodder, more than doubled in weight; gaining 133.3% of the original weight. In contrast, the other fish species, fed on mixed mangrove-commercial diets, showed a gain of 75.4% for 40% *Avicennia* and only 43.9% for 40% *Rhizophora*. The 75.4% weight gain of fish fed on 40% *Avicennia* corresponds closely to the remaining 60% of the commercial portion in the control diet (*i.e.* 100% – 40%). This fact can be readily seen from a comparison of the latter weight gain with 60% of the weight gain recorded for the control species. The comparison indicates that the fish species on the 40% *Avicennia* diet did not benefit at all from the nutrients present in the *Avicennia* leaves, despite their reasonably high calorific values. The inaccessibility of these nutrients to the feeding fishes is apparently caused by the toughness of the cell walls of the leaves. On the other hand, the 43% weight gain of the fish feeding on the 40% *Rhizophora* diet is sizably lower than an equivalent weight gain due to feeding on the 60% commercial diet alone, which should correspond to about 60% of the weight gain of the control species as was the case with *Avicennia*. It was thus suspected that the leaves of *Rhizophora* species, apart from being tough, may contain some leachable material that can possibly inhibit the action of some digestive enzymes or retard the metabolism or assimilation of food.

At the outset of the nutrition experiments, food was provided to fishes in quantities equivalent to 4% of the fish weight. This quantity was found insufficient to cover the fish maintenance requirement. Over a period of only one week's time, fishes in both of the 80% treatments started to lose weight and so the food ratio was raised to 6% of the fish weight. At this 6% level, the 80% *Avicennia* fishes barely maintained their weight (making only a negligible 1.6% gain) whereas the 80% *Rhizophora* continued to lose weight. Also, during the course of the nutrition experiments, a direct proportionality was observed between the per cent of mangrove supplement in the diet and the amount of fecal matter excreted by the feeding fishes. The fecal matter increased with increasing mangrove per cent in the diet. These observations are consistent with the presumed indigestibility of mangrove leaves. In contrast, control fishes feeding on pure commercial fodder did not experience the latter phenomenon.

Although the presence of even trace amounts of certain elements in the feed can lead to serious toxicity problems, there are several other elements whose availability to the feeder is considered of dire importance from a nutrtional point of view. Deficiency in one or more of such elements can lead to a variety of problems including

Blauth, O.J., Charezinski, M. and **Berbec, H.** (1963) A new rapid method for determination of tryptophan. *Analytical Biochem.* **6:** 69-70.

Block, R.J., Durrum, E.L. and **Zwey, G.** (1958) *A Manual of Paper Chromatography and Paper Electrophoresis.* 2nd Ed., New York, Academic Press, pp. 75-80.

Cole, W.H. (1950) *Co-operative Determination of the Amino Acid Content and the Nutritive of Six Selected Protein Food Sources,* Ph.D. Thesis, Rutgers University, New Brunswick, New Jersey.

David, J. Holme and **Hazel, Peck** (1983) *Analytical Biochemistry,* Longman, London and New York, 428 p.

Davis, B.J. (1984) Disc electrophoresis method and application to human serum protein. *Ann. Acad. Sci.* **2:** 404-427.

Edwards, F.J. (1987) Climate and Oceanography. *In:* **Edwards, A.J.** and **Head, S.M.** (eds.) *Red Sea,* London, Pergamon Press, pp. 46-68.

FAO/WHO (1973) *Report of joint FAO/WHO committee. Energy and protein requirements.* WHO Tech. Rep. Ser. No. 522, Geneva, World Health Organization.

Fröstner, U. and **Patchineelam, S.R.** (1980) Chemical association of heavy metals in polluted sediments from the lower Rhine. *In:* Particulates in Water "Advances in Chemistry", *American Chemical Society* **189:** 177-193.

Halver, J.E. (1972) *Fish Nutrition,* New York, Academic Press.

Heilmann, J., Barollier, J. and **Watzke, E.** (1957) New ninhydrin method for the analysis of amino acids. *Z. Physiol. Chem.,* **309,** 219.

Hickling, C.F. (1971) *Fish Culture, 2nd* ed., London, Faber and Faber.

Jeng, S.S. and **Sun, L.T.** (1981) Effects of dietary zinc levels on zinc concentration in tissues of common carp. *J. Nut.* **111:** 134-140.

Khafaji, A.K., Mandura, A.S. and **Saifullah, S.** (1988) Temporal and spatial variations in the carbohydrate, lipid and protein contents of *Avicennia marina* from the Saudi Arabian Red Sea coast. *Proc. Saudi Biol. Soc.* Vol. **3.**

Khar, N.D. (1954) *Sci. Cult.* **19,** 556.

Khar, N.D. and **Negi, S.S.** (1953) *Sci. Cult.* **18,** 232.

Kuiken, K.A., Lyman, G.M. and **Halle, F.** (1947) Factor which influence the stability of tryptophane during the hydrolysis of proteins in alkaline solution. *J. Biol. Chem.* **171:** 551-560.

Levy, A.L. and **Chung, D.** (1953) Two dimensional separation of amino acids. *An. Chem.* **25,** 396.

Mandura, A.S., Saifullah, M.S. and **Khafaji, A.K.** (1987) Mangrove ecosystem of southern Red Sea coast of Saudi Arabia. *Proc. Saudi Biol. Soc.* **10:** 165-193.

Mirza, I.L. and **Cresser, M.S.** (1985) *Environmental Chemical Analysis,* New York, International Textbook Co.

Norman, Helen, A., McMillan, Calvin and **Thompson, Guy A. Jr.** (1984) Phosphatidylglycerol Molecular Species in Chilling-sensitive and Chilling Resistant Populations of *Avicennia germinans* (L.) L. *Plant Cell Physiol.* **25**(8): 1437-1444.

Por, F.D. and **Dor, I.** (1975) The hard bottom mangroves of Sinai: Limits of an ecosystem. *Rapp. Common. Int. Mar. Medit.* **23**(27): 144-145.

Por, F.D., Dor, I. and **Amir, A.** (1977) The mangal of Sinai: Limits of an ecosystem. *Helgal. Wiss. Meersunters* **30:** 295-314.

Qasim, R., Barakati, S., Ahmed, S. and **Ilyas, M.** (1986) Biochemical composition of the mangrove, *Avicennia marina,* foliage, *Pakistan J. Sci. Ind. Res.* **29,** 1.

Roland, J.F. and **Gross, A.H.** (1954) Amino acids analysis in B. lactoglobulin by paper chromatography method. *Anal. Chem.,* **26,** 502.

Shallan, M.A.M. (1984) *Biochemical studies and production of proteins from natural products,* M.Sc. Thesis, Cairo University, pp. 33-58.

Sokoloff, B., Redd, J.B. and **Butschar, R.Q.** (1950) Nutritive value of mangrove leaves. *J. Fla. Acad. Sci.,* **12,** 191.

Talfan, I.A. and **Salman, A.Z.** (1982) *Fish Food and Nutrition.* Al Basra Universal Journal, Ministry of High Education and Scientific Research.

دراسة فايتوكيميائية لنبات المانجروف ومدى صلاحية إضافته للأعلاف

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المستخلص . تشمل هذه الدراسة المكونات الكيميائية لنباتي المانجروف أفيسينيا مارينا و ريزوفورا ميكروناتا (أوراق - سوق - جذور) فيما يتعلق بالبروتينات والأحماض الأمينية والدهنية والعناصر المعدنية باستخدام الأجهزة المختلفة وذلك لبيان مدى إمكانية إضافة المانجروف إلى علائق التغذية . وتشمل هذه الدراسة أيضًا تجارب التغذية التي أجريت لمعرفة استخدام نسب مختلفة من أوراق نوعي المانجروف على نمو الأسماك . وقد روعى في تكوين العلائق الخاصة بمعاملات تجارب التغذية نتائج التحاليل الخاصة بالمكونات وكذا القيمة الحرارية الكلية للعليقه . وقد أثبتت النتائج عمومًا ارتفاع نسب البروتينات والكربوهيدرات والليبيدات والدهون نسبيًا في أوراق نباتات المانجروف مما شجع على قصر تجارب التغذية على الأوراق فقط . ومع أن نتائج تجربة التغذية كانت سلبية كما هو متوقع بالنسبة للمعاملات التي استخدمت فيها أوراق النباتات وخصوصًا الريزوفورا مقارنة بالكونترول إلا أنه لم تكن هناك آثار بيولوجية عكسية على الأسماك التي استخدمت في التجربة . وأشار الباحثون إلى أن هذه النتيجة قد ترجع إلى صلابة جدر الخلايا بالاضافة إلى وجود عوامل مثبطة إمّا لإنزيمات الهضم أو التمثيل الغذائي أو الامتصاص بالقناة الهضمية ضمن مكونات أوراق المانجروف .