

The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (<http://www.aquaculturehub.org>) members and registered individuals and institutions. Please visit our website (<http://siamb.org.il>) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Editor-in-Chief

Dan Mires

Editorial Board

Sheenan Harpaz Agricultural Research Organization
Beit Dagan, Israel

Zvi Yaron Dept. of Zoology
Tel Aviv University
Tel Aviv, Israel

Angelo Colorni National Center for Mariculture, IOLR
Eilat, Israel

Rina Chakrabarti Aqua Research Lab
Dept. of Zoology
University of Delhi

Ingrid Lupatsch Swansea University
Singleton Park, Swansea, UK

Jaap van Rijn The Hebrew University
Faculty of Agriculture
Israel

Spencer Malecha Dept. of Human Nutrition, Food
and Animal Sciences
University of Hawaii

Daniel Golani The Hebrew University of Jerusalem
Jerusalem, Israel

Emilio Tibaldi Udine University
Udine, Italy

Copy Editor

Ellen Rosenberg

Published under auspices of
**The Society of Israeli Aquaculture and
Marine Biotechnology (SIAMB),
University of Hawaii at Manoa Library**

and
**University of Hawaii Aquaculture
Program** in association with
AquacultureHub

<http://www.aquaculturehub.org>



UNIVERSITY
of HAWAII
MĀNOA
LIBRARY



AquacultureHub
educate • learn • share • engage

ISSN 0792 - 156X

© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL

Phone: + 972 52 3965809

<http://siamb.org.il>

CARBOHYDRASES IN THE ALIMENTARY CANAL AND ASSOCIATED ORGANS OF THE AFRICAN SNAKEHEAD, *PARACHANNA AFRICANS* (OSTEICHTHYES: CHANNIDAE)

Ovie Kori-Siakpere*

Department of Zoology, Delta State University, P.M.B. 1, Abraka, Nigeria

(Received 22.10.02, Accepted 25.7.03)

Key words: African snakehead, amylase, carbohydrases, lactase, maltase, *Parachanna africans*, sucrase

Abstract

Standard qualitative and quantitative methods were employed to determine the presence and activity of digestive carbohydrases in different regions of the alimentary canal and associated organs of the African snakehead, *Parachanna africans*. Qualitatively, amylase, maltase, sucrase, and lactase were present in the stomach, pyloric ceca, anterior and posterior intestine, liver and spleen. No carbohydrases were found in the esophagus whereas cellulase was completely absent throughout the canal and organs. Quantitatively, a relatively large amount of lactase and maltase was found throughout the canal and organs (except the esophagus) while sucrase was active in varying degrees in all the canal regions and associated organs (except the esophagus).

Introduction

Reports on several marine and freshwater fishes have shown that relative activities of digestive enzymes correlate with the nature and composition of the food consumed (Al-Hussaini, 1949; Fish, 1960; Cockson and Bourne, 1972; Jonas et al., 1983; Olatunde et al., 1991) and that there are seasonal variations in digestive enzyme activity (Ananichev, 1959; Gelman et al., 1984). Sucrase, maltase,

lactase and amylase are among the carbohydrate-digesting enzymes in various species of fish (Phillip, 1969). Olatunde and Ogunbiyi (1977), working on freshwater species from Lake Kainji, Nigeria, reported the presence of amylase activity in the tropical catfish *Entropicus niloticus* and *Schilbe mystus* but were unable to detect any cellulase, lactase, sucrase or maltase activity in these species.

* E-mail: oviekori@yahoo.com

Akintunde (1984) recorded enzymes capable of digesting carbohydrates, proteins and lipids in different sections of the gut of *Sarotherodon galilaeus* and that the enzymatic activity correlated with the diet of the fish. Other works include those on *Clarias lazera* (Olatunde et al., 1988), *Sarotherodon melanotheron* (Ugwumba, 1989), *Heterotis niloticus* (Ugwumba, 1993) and other freshwater fish (Ugwumba et al., 1998), which reported the presence of amylase and maltase and the absence of sucrase, lactase and cellulase. In contrast, Sarbahi (1951) reported sucrase and maltase activity in the goldfish, *Carassius auratus*.

This work continues earlier investigations of digestive enzymes in commercial tropical freshwater fishes such as *Clarias anguillaris* (Kori-Siakpere, 1999) and *Clarias buthupogon* (Kori-Siakpere, 2000). This paper focuses on carbohydrases in the alimentary canal and associated organs of the African snakehead, *Channa africans*. Snakeheads are very common in the swamps of the Niger Delta, Nigeria, and are cherished by consumers (Alfred-Ockiya, 1998). Fish farmers are beginning to include them in polyculture systems as 'police fish' to control the over-population of tilapia (Wee, 1982). Fagbenro (1989) used *Channa obscura* for the control and production of *Tilapia guineensis* in a freshwater semi-intensive culture system. The implication is that the demand for snakehead will increase, and the need for its management is obvious. Hopefully, the obtained results will contribute to the understanding of carbohydrate digestion and aspects of nutritional physiology in *Parachanna africans*.

Materials and Methods

Fish. Live specimens of the African snakehead, *P. africans*, were obtained from a local fish market, transported to the laboratory in their natural water and kept in plastic basins. The fish ranged 14-28.5 cm (average 17.75 cm) and 88.5-105.9 g (average 94.5 g). They were maintained in the laboratory for a minimum of 48 hours during which they were not fed, to clear the alimentary canal of extraneous materials and bring them to approximate-

ly similar physiological conditions for the enzyme assays. Fish of both sexes were used without discrimination and all were considered normal on the basis of their healthy-looking appearance and absence of obvious signs of disease.

Preparation of the enzyme extract. After preliminary examination of length and weight, the fish were sacrificed. The entire alimentary canal and associated organs were promptly dissected out and placed on ice. The alimentary canal was cut into portions: the esophagus, stomach, pyloric caeca, anterior intestine and posterior intestine. The liver and spleen were similarly dissected out. All samples were flushed with normal saline (0.85% NaCl), weighed and then homogenized with ten parts of iced distilled water to produce a 1:10 homogenate which was refrigerated overnight to ensure complete extraction of the enzymes. The homogenate was then centrifuged for 15 min at 4800 rpm. The supernatant was strained through glass wool to remove the lipids; the clear supernatant was used as the source of enzyme activity without further purification and kept refrigerated at -4°C. Bile was used directly after 1:10 dilution as the source of enzyme activity.

Assay for carbohydrases. Amylase, maltase, lactase, sucrase and cellulase were assayed. Various carbohydrates were used as substrates to assay the carbohydrases. A freshly prepared solution of 1% starch in 0.1 M sodium acetate buffer at pH 5.0 was used for amylase, a 1% sucrose solution in 0.1 M acetate buffer at pH 5.0 was used for sucrase, a 1% lactose solution in 0.2 M phosphate buffer at pH 7.0 was used for lactase, a 1% maltose solution in 0.2 M phosphate buffer at pH 7.0 was used for maltase and a 1% solution of carboxymethyl cellulose in 0.1 M acetate buffer at pH 5.0 was used for cellulase.

One ml of the crude enzyme extract was added to 1 ml of the appropriate buffered substrate and incubated in a water bath at 35°C for 24 hours. After equilibration, the reaction was started by adding 2.0 ml of the crude enzyme extract. Simultaneous controls contained denatured (boiled) enzyme extract or distilled water and the substrate instead of the

enzyme extract. After incubation, digestion was stopped by placing the test mixture in boiling water for 1 min. Qualitative determination of carbohydrases was carried out by heating samples with 5 ml of Benedict's qualitative reagent for 15 min. The solution was cooled and color changes were observed. A positive result was indicated by a brick-red color or formation of a colored precipitate. Quantitatively, the presence of free-reducing sugars in the enzyme extract was determined using the colorimetric estimation of reducing sugar by the dinitrosalicylate (DNS) method of Plummer (1978). The presence of free-reducing sugars was determined and the amounts of DNS reduced in the presence of the substrates and extracts were compared. One unit of carbohydrase activity was defined as the amount of enzyme that caused an increase in absorbance at 540 nm of 0.001 nm per min on a spectrophotometer. Enzyme activity values are averages of five enzyme preparations for each region of the alimentary canal or associated organ.

Results

Qualitative distribution of carbohydrase activity. The hydrolytic activities determined by Benedict's qualitative test are shown in Table 1. No carbohydrase activity was recorded in the esophagus. Amylase activity was weak

throughout the alimentary canal and associated organs. Maltase activity was strong only in the posterior intestine and liver. Lactase activity was weak in the stomach, but strong in the rest of the canal sections and organs. Sucrase activity ranged from very weak in the stomach and pyloric ceca to fairly strong in the anterior intestine, posterior intestine and spleen. Cellulase activity was completely absent in all regions of the alimentary canal and associated organs.

Quantitative distribution of carbohydrase activity. The quantitative carbohydrase activity is presented in Table 2. Relative activity for the canal sections and organs and for the carbohydrases is presented in Figs. 1 and 2, respectively. Fig. 1 shows the relative amount of each carbohydrase in each section or organ compared to the total of that carbohydrase in the system. Fig. 2 shows the relative amount of each carbohydrase in each section or organ compared to the total carbohydrases in that section or organs.

Discussion

Results indicate that carbohydrase activity in *P. africans* varies between regions of the alimentary canal and associated organs, with no activity in the esophagus. This can be expected because, in most animals, the esophagus does not perform the function of digestion, it

Table 1. Qualitative distribution of carbohydrase activity in the alimentary canal and associated organs of *Parachanna africans*.

Carbohydrase	Esophagus	Stomach	Pyloric ceca	Anterior intestine	Posterior intestine	Liver	Spleen
Amylase	-	+	±	±	±	±	±
Maltase	-	±	+	+	++	++	+
Lactase	-	+	++	++	++	++	+++
Sucrase	-	±	±	++	++	+	++
Cellulase	-	-	-	-	-	-	-

- no activity, ± very weak activity, + weak activity, ++ fairly strong activity, +++ strong activity

Table 2. Quantitative distribution of carbohydrase activity* in the alimentary canal and associated organs of *Parachanna africans*.

Carbohydrase	Esophagus	Stomach	Pyloric ceca	Anterior intestine	Posterior intestine	Liver	Spleen
Amylase	-	0.25	0.15	0.16	0.18	0.17	0.08
Maltase	-	0.13	0.43	0.46	0.50	0.53	0.34
Lactase	-	0.22	0.45	0.55	0.57	0.45	0.78
Sucrase	-	0.09	0.12	0.53	0.48	0.37	0.50
Cellulase	-	-	-	-	-	-	-

* = Δ nm/min, measured by spectrophotometer

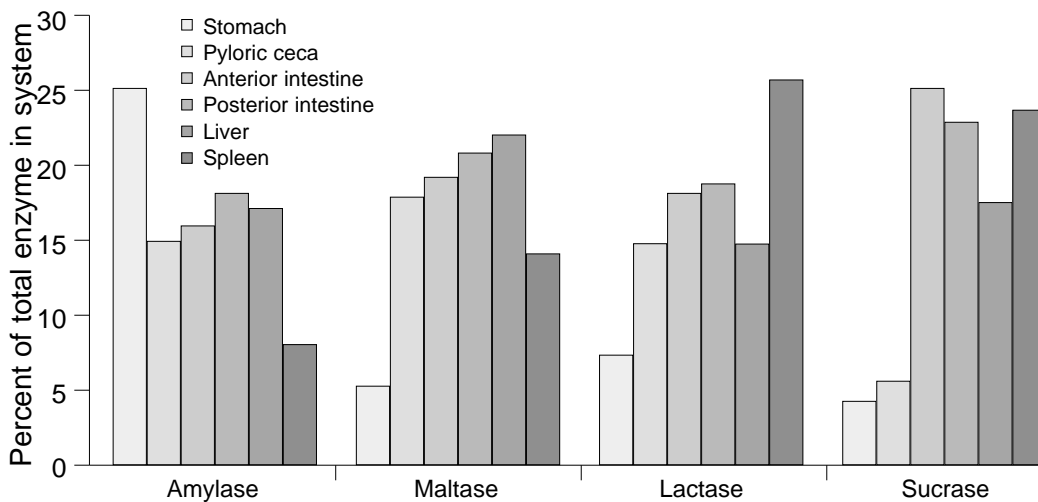


Fig. 1. Percent of the total respective carbohydrase found in each section of the alimentary canal and associated organ in *Parachanna africans*.

being just a passageway between the buccal cavity and the stomach. The fact that food is not stored or kept there means that the presence of enzymes in this region is unnecessary.

The distribution of carbohydrases along the gut of this fish corresponds with that of other animals including fishes. Amylase activ-

ity was found in the stomach, indicating that starch digestion begins in the stomach and continues downward in the other parts of the alimentary canal. Similar results were reported by Fange and Grove (1979) and Fraisse et al (1981), who showed that digestion of starch begins in the stomach and that there is a decreasing gradient towards the posterior

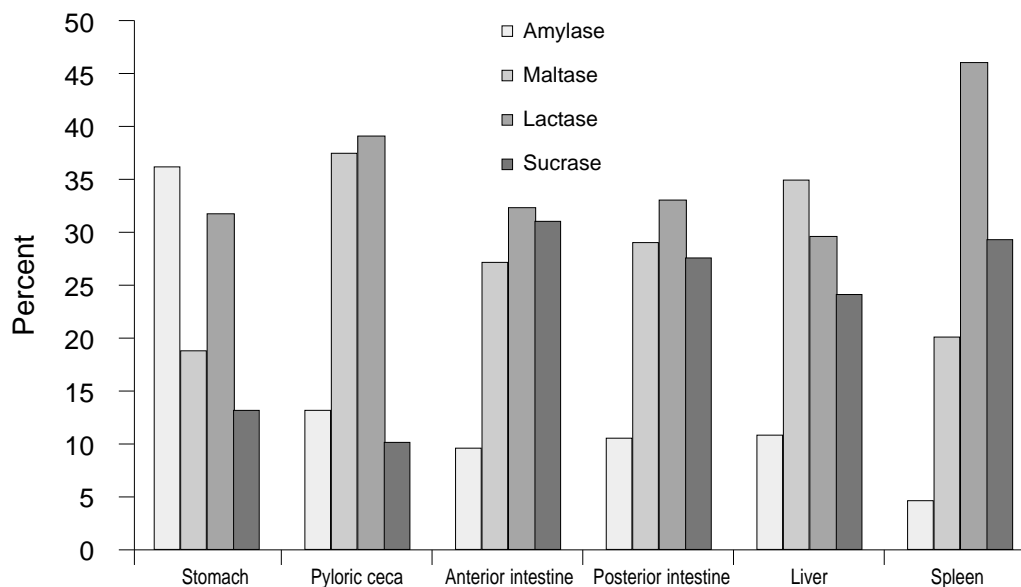


Fig. 2. Percent of each enzyme in alimentary canal sections and associated organs in *Parachanna africans*.

parts of the intestine. This trend is referred to as the descending proximo-distal gradient (Kuz'mina, 1985).

Maltase activity was detected throughout the alimentary canal, with the posterior intestine and liver having the highest activity. Maltase activity in these sites indicates that the snakehead must cope with a high level of dietary carbohydrates and ensures that dietary starch and maltose are efficiently and completely hydrolyzed to glucose. Maltase activity has been reported in *Carassius auratus* (Sarbah, 1951), *Cyprinus carpio*, *Pagrus major* and *Plecoglossus altivelis* (Kawai and Ikeda, 1971), *Chanos chanos* (Chiu and Benitez, 1981), *Sarotherodon melanotheron* (Ugwumba, 1989), *Heterotis niloticus* (Ugwumba, 1993) and other freshwater fish species (Ugwumba et al., 1998).

Lactase recorded the highest activity among all the carbohydrases studied, with the highest value in the spleen. Lactase activity was low in the liver, pyloric ceca and intestines and lowest in the stomach. The

presence of lactase is an important revelation since teleost do not normally consume milk, which is the main substrate for lactase. Lagler et al. (1977) reported the presence of lactase in the pyloric ceca of the trout while Olatunde et al. (1988) reported the presence of lactase in the intestine of *Clarias lazera*. Similarly, the presence of lactase in the alimentary canals of *Clarias anguillaris* (Kori-Siakpere, 1999) and *Clarias buthupogon* (Kori-Siakpere, 2000) was reported. Olatunde et al. (1991) attributed the presence of lactase in the gut of fish to the possibility that there are materials in algae, diatoms and desmids, normally eaten by fish, that have a structure similar to lactose

Sucrase activity was strong in the anterior and posterior intestine and the spleen. Sucrase activity was not detected in earlier works such as Olatunde and Ogunbiyi (1977), Akintunde (1984), Ugwumba (1989, 1993), Ugwumba et al (1998) and Kori-Siakpere (1999, 2000). According to Olatunde and Ogunbiyi (1977), sucrase is one of the enzymes not normally found in teleosts, prob-

ably due to the absence of the substrate for the enzyme in the usual fish diet. However, Chiu and Benitez (1981) reported sucrase activity in the milkfish *Chanos chanos*.

The only carbohydrase not recorded in this study was cellulase. No trace of cellulase was found in the alimentary canal and associated organs of the experimental fish. This is in agreement with the observations of most researchers working on indigenous cellulase of fish. No cellulase activity was detected in *Tilapia mossambica* (Fish, 1960), *Salmo gairdneri* (Kitamikado and Tachino, 1960), *Sarotherodon melanotheron* (Ugwumba, 1989), *Heterotis niloticus* (Ugwumba, 1993) and other freshwater fish species (Ugwumba et al., 1998). Yokoe and Yasumasu (1964) and Stickney and Shumway (1974) indicated that fishes as well as other chordates do not produce enzymes that hydrolyze cellulose and other polysaccharides of high molecular weight. Instead, the cellulose in the intestinal tract of chordates is decomposed by microorganisms and other invertebrates capable of digesting cellulose in the gut of fish. It has also been suggested that the evolutionary development of vertebrates is accompanied by a reduction or loss of cellulase activity (Yokoe and Yasumasu, 1964).

The presence and activity of these carbohydrases in the alimentary canal and associated organs of *P. africans* shows that the fish is well adapted to and capable of digesting the plant component of its diet. The amount of activity also shows the proportion of carbohydrates included in its diet. Based on the distribution of the various carbohydrases, it could be said that its food contains a high proportion of lactose, followed by maltose, sucrose and amylose, in descending order. On the whole, the generally low activity of carbohydrases in this fish indicates its carnivorous mode of nutrition, feeding almost exclusively on fishes and frogs.

References

- Akintunde E.A.**, 1984. Digestive enzymes in the gut of *Sarotherodon galileus*, syn. *Tilapia galilaea* (Fam. Cichlidae) of Lake Kainji, Nigeria. *Nigerian J. Sci.*, 18:22-25.
- Alfred-Ockiya J.F.**, 1998. Organoleptic evaluation and acceptability of four traditionally smoked dried freshwater fishes in River State, Nigeria. *Global J. Pure Appl. Sci.*, 4(4):357-360.
- Al-Hussiani A.H.**, 1949. On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits. *Q. J. Micro. Sci.*, 90:323-324.
- Ananichev A.B.**, 1959. Digestive enzymes of fish and seasonal changes in their activity. *Biochemistry* (Moscow), 24:1033-1040.
- Chiu Y.N. and L.Y. Benitez**, 1981. Studies on the carbohydrases in the digestive tract of milkfish, *Chanos chanos*. *Mar. Biol.*, 61:247-254.
- Cockson A. and D. Bourne**, 1972. Enzymes in the digestive tract of two species of euryhaline fish. *Comp. Biochem. Physiol.*, 41A:715-718.
- Fagbenro O. A.**, 1989. Recruitment control and production of tilapia. *J. Aquatic Sci.*, 4:7-10.
- Fange R. and D. Grove**, 1979. Digestive enzymes. In: W.S. Hoar, D.J. Randall, J.R. Brett (eds.). *Fish Physiology*, Vol. 8. Academic Press, New York.
- Fish G.R.**, 1960. The comparative activity of some digestive enzymes in the alimentary canal of tilapia and perch. *Hydrobiologia*, 15:161-178.
- Fraisse M., Woo N.Y.S., Hoiallae-Epeyre J. and J.C. Murat**, 1981. Distribution pattern of digestive enzyme activities in the catfish (*Ameiurus nebulosus* L.) and the carp (*Cyprinus carpio* L.). *Comp. Biochem. Physiol.*, A70:443-446.
- Gelman A., Mokady S. and U. Cogan**, 1984. The effect of seasonal changes in the activity of intestinal alkaline phosphatase of pike perch, *Lucioperka lucioperka* and of bream, *Abramis brama*. *J. Fish Biol.*, 25:207-212.
- Jonas E., Ragyanskil M., Olah J. and J. Boross**, 1983. Enzymes of carnivorous (*Silurus glanis* L.), herbivorous (*Hypophthalmichthys molitrix* val) and omnivorous (*Cyprinus carpio* L.) fishes. *Aquaculture*, 30:145-154.
- Kawai S. and S. Ikeda**, 1971. Studies on digestive enzymes of fishes. I. Carbohydrases in digestive organs of several fishes. *Bull. Jpn. Soc. Sci. Fish.*, 37:333-337.

- Kitamikado M. and S. Tachino**, 1960. Studies on the digestive enzymes of the rainbow trout. I. Carbohydrases. *Bull. Jpn. Soc. Sci. Fish.*, 26:679-684.
- Kori-Siakpere O.**, 1999. Digestive enzymes in the alimentary canal of *Clarias anguillaris* (Pisces:Clariidae). *Trop. Freshwater Biol.*, 8:55-63.
- Kori-Siakpere O.**, 2000. Digestive enzymes in the gastrointestinal tract of the African mudfish: *Clarias buthupogon* (Pisces:Clariidae). *Biosci. Res. Comm.*, 12(3):303-308.
- Kuz'mina V.V.**, 1985. Distribution of carbohydrases activity along the intestine of several species of freshwater fish. *J. Ichthyol.*, 25(4):137-143.
- Lagler K.F., Bardach J.E., Miller R.R. and D.R.M. Passino**, 1977. *Ichthyology*, 2nd ed. John Wiley and Sons, New York.
- Olatunde A.A. and O.A. Ogunbiyi**, 1977. Digestive enzymes in the alimentary tracts of three tropical catfish. *Hydrobiologia*, 56:21-24.
- Olatunde A.A., Ukoha A.I. and B.F. Oguntayo**, 1988. Digestive enzymes in the alimentary tract of *Clarias lazera* Cuvier and Vallenciennes, family Clariidae (Osteichthyes, Siluriformes). *Arch. Hydrobiol.*, 112:107-113.
- Olatunde A.A., Ukoha A.I. and P.K. Singh**, 1991. The relationship between feeding habits and digestive carbohydrases of three tropical freshwater fishes. *J. Aquatic Sci.*, 6:1-6.
- Phillip A.M. Jr.**, 1969. Nutrition, digestion and energy utilization. In: W.S. Hoar and D.J. Randall (eds.). *Fish Physiology*, Vol. 1. Academic Press, New York.
- Plummer D.T.**, 1978. *An Introduction to Practical Biochemistry*, 2nd ed. McGraw Hill Book Co., UK.
- Sarbahi D.S.**, 1951. Studies on the digestive tract and digestive enzymes of the goldfish *Carassius auratus* (Linn) and the largemouth blackbass, *Micropterus salmoides*. *Biol. Bull.*, 66:244-257.
- Stickney R.R. and S.E. Shunway**, 1974. Occurrence of cellulase activity in the stomach of fishes. *J. Fish Biol.*, 6:779-790.
- Ugwumba A.A.A.**, 1989. Carbohydrases in the digestive tract of cichlid fish *Sarotherodon melanotheron* (Rupell) in Awba Lake, Ibadan, Nigeria. *J. Exp. Appl. Biol.*, 1:45-50.
- Ugwumba A.A.A.**, 1993. Carbohydrases in the digestive tract of the African bony-tongue *Heterotis niloticus* (Pisces:Osteoglossidae). *Hydrobiologia* 257:95-100.
- Ugwumba A.A.A., Ugwumba A.O., Jimoh M. and D. Adegbola**, 1998. Carbohydrases in the digestive tract of *Oreochromis niloticus* (Linnaeus), *Sarotherodon galilaeus* (Artemis), *Tilapia zilli* (Gervais), *Clarias gariepinus* (Burchell) and *Chrysichthys nigrodigitatus* (Lacepede). pp. 123-130. In: *Sustainable Utilisation of Aquatic/Wetland Resources - Selected Papers from 9th/10th Annu. Conf. Nigerian Assoc. for Aquatic Sci.*, November 30-December 2, 1995, Abeokuta.
- Wee K. L.**, 1982. Snakeheads - their biology and culture. In: J.F. Muir and R.J. Roberts (eds.). *Recent Advances in Aquaculture*. Westview Press Boulder, Colorado.
- Yokoe Y. and I. Yasumasu**, 1964. The distribution of cellulase in invertebrates. *Comp. Biochem. Physiol.*, 13:323-333.