

Bazyli CZECHUGA¹, Bernard KŁYSZEJKO²

Carotenoids in fish

CAROTENOID CONTENT IN THE FRY OF AFRICAN SHARPTOOTH
CATFISH (*CLARIAS GARIEPINUS* BURCHELL) *ZASOBNOŚĆ W KAROTENOIDY NARYBKU SUMA AFRYKAŃSKIEGO
(*CLARIAS GARIEPINUS* BURCHELL)¹Department of General Biology, Academy of Medicine, Białystok, Poland²Department of Fish Physiology, University of Agriculture, Szczecin, Poland

Using column and thin layer chromatography, carotenoid contents were determined in the African sharptooth catfish (*Clarias gariepinus* Burchell).

INTRODUCTION

A permanent protein deficit in human nutrition in Africa demands that the international community seek to at least partially ameliorate the problem by looking for new species amenable to mass culture. Among African species, the sharptooth catfish was chosen due to particulars of its biology (Richter, 1976; Viveen et al., 1985, 1986). The species occurs throughout the continent, for which reasons individuals were erroneously described four times as separate species, although they belonged to the same one (Van der Waal, 1974). The species is not selective in terms of its habitat preference and is thus found in all types of natural water bodies of Africa and even in open sewage canals. Owing to its ability to use atmospheric oxygen for respiration, the species is able to live in intermittent water bodies (Babiker, 1984). *C. gariepinus* tolerates high contents of dissolved carbon dioxide, nitrites, and nitrates and is resistant to a wide range of temperature (Britz and Hecht, 1987) and salinity (Czerwiński, 1984) variation. The fish is omnivorous and feeds both on plants and animals, primarily other fish, live and dead. As it uses its olfactory and tactile organs in looking for food, the species feeds successfully in turbid, filthy water. Such biological properties of *C. gariepinus* resulted in its being an object of interest for cage cultures in heated water discharged by power stations outside Africa.

* Part 51 in the series "carotenoids in fish."

In this context we became interested in the content of carotenoids, i.a. as a vitamin A source, in the species.

MATERIALS AND METHODS

Assays were made on 5-month-old individuals of *C. gariepinus*, obtained from a heated water culture facility at Nowe Czarnowo near Szczecin, the water being discharged by the Dolna Odra power station.

Analyses were made on skin (from 7 individuals), muscles (3 individuals), intestine (14 individuals), and liver (27 individuals). Samples ranged in weight from 15.17 (skin) to 16.94 (liver) g wet weight.

Each sample was homogenized and placed in a dark bottle with 95% acetone; the samples were kept refrigerated until analysed. The carotenoid pigments were separated by column and thin layer chromatography; the techniques were described in detail in a previous paper (Czczuga and Czerpak, 1976). Prior to the analysis the samples were hydrolysed for 24 h in 10% KOH in nitrogen at room temperature. The hydrolysate was transferred to an Al₂O₃-filled column (Quickfit, England). The column length ranged from 15 to 25 cm. The fractions were elued with different solvent systems (Czczuga and Czerpak, 1976).

Apart from column chromatography, the acetone extract obtained was separated into fractions with thin layer chromatography on silica gel-covered glass plates; various solvent systems were used as well (Czczuga and Czerpak, 1968). The R_f values were determined according to the generally accepted principles.

The carotenoids were identified based on: a) column chromatograms; b) absorption maxima of pigments in different solvents; c) epi- to hypophase ratio in hexane and 95% methanol; d) comparison of thin layer chromatography R_f values with standards (Hoffman-La Roche and Co., Basel, Switzerland and Sigma Chemical Company, USA) for β-carotene, β-cryptaxanthin, lutein, canthaxanthin, β-doradexanthin, and astaxanthin; e) the presence of allohydroxy groups determined with acidic chloroform; f) epoxy test. Carotenoid contents were determined based on quantitative aspects of absorption. The assays were based on extinction coefficients (E, 1%/cm) at appropriate absorption peaks in petroleum benzine or hexane (Davies, 1976). The chemical structure of different carotenoids is presented following Straub (1987).

RESULTS

The presence of a total of 13 carotenoids was found in *C. gariepinus* individuals (Table 1, Fig. 1). The skin and muscles contained 6 carotenoids each, while the intestine and liver contained 9 carotenoids each (Table 2). Taraxanthin was found to be the dominant carotenoid in the skin, muscles, and intestine, while astaxanthin was present in the highest contents in the liver. The contribution of ketocarotenoids ranged from trace amounts (skin) to 71.7 % in the liver. The liver was the carotenoid–richest organ of *C. gariepinus* (0.879 $\mu\text{g/g}$ w.w.), the other ones containing much lower contents (from 0.328 $\mu\text{g/g}$ w.w. in muscles to 0.379 $\mu\text{g/g}$ w.w. in the intestine). The highest contents of those carotenoids forming provitamin A were found in the liver and intestine, while the skin and muscles were less enriched (Table 3).

Table 1

Carotenoids found in *Clarias gariepinus*

No.	Carotenoid	Structure (see Fig. 1)	Semisystematic name
1	β -carotene	A - R - A	β,β -Carotene
2	β -cryptoxanthin	A - R - C	β,β -Caroten-3-ol
3	lutein	C - R - D	β,ϵ -Carotene-3,3'-diol
4	tunaxanthin	D - R - D	ϵ,ϵ -Carotene-3,3'-diol
5	taraxanthin	D - R - G	5,6-Epoxy-5,6-dihydro- β,ϵ -carotene-3,3'-diol
6	antheraxanthin	C - R - G	5,6-Epoxy-5,6-dihydro- β,β -carotene-3,3'-diol
7	hydroxyechinenone	A - R - F	3-Hydroxy- β,β -caroten-4-one
8	canthaxanthin	E - R - E	β,β -Carotene-4,4'-dione
9	α -doradexanthin	D - R - F	3,3'-Dihydroxy- β,ϵ -caroten-4-one
10	β -doradexanthin	C - R - F	3,3'-Dihydroxy- β,β -caroten-4-one
11	astaxanthin	F - R - F	3,3'-Dihydroxy- β,β -carotene-4,4'-dione
12	violaxanthin	G - R - G	5,6,5',6'-Diepoxy-5,6,5',6'-tetrahydro- β,β -carotene-3,3'-diol
13	mutatoxanthin	C - R ₁ - H	5,8-Epoxy-5,8-dihydro- β,β -carotene-3,3'-diol

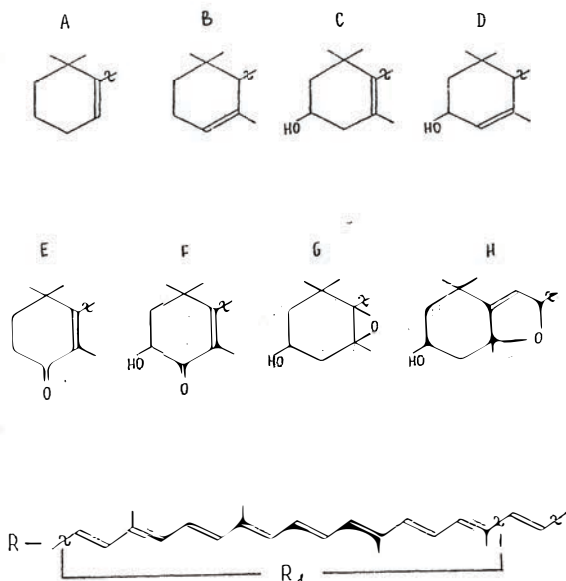


Fig. 1. Structural features of carotenoids

Table 2

Carotenoid distribution in *Clarias gariepinus*

Part of body	Carotenoid (see Table 1 and Fig. 1)	Major carotenoid (%)	Ketocarotenoids ^x (%)	Total carotenoid content ($\mu\text{g g}^{-1}$ w.w.)
Skin	1, 4, 5, 6, 12, 13	5 (46.8)	trace	0.394
Muscles	1, 4, 5, 9, 10, 13	5 (42.1)	24.9	0.328
Intestine	1, 2, 3, 4, 5, 8, 9, 10, 12	5 (28.9)	21.0	0.397
Liver	1, 3, 5, 6, 7, 8, 9, 10, 11	11 (20.2)	71.7	0.879

^x - hydroxyechinenone, canthaxanthin, α - and β -doradexanthin and astaxanthin

Table 3

Contents of carotenoids - provitamin A in *Clarias gariepinus*

Part of body	Total carotenoid content ($\mu\text{g g}^{-1}$ w.w.)	Provitamin A	
		$\mu\text{g g}^{-1}$ w.w.	%
Skin	0.394	0.026	6.7
Muscles	0.328	0.018	5.6
Intestine	0.397	0.054	13.7
Liver	0.879	0.067	7.6

DISCUSSION

Due to an artificial, and most often uniform diet in mass fish cultures, the fish meat shows worsened flavour qualities and differs in colour from meat obtained from fish living under natural conditions. To improve the market value of fish meat, feeds are frequently enriched with colorants, i.e., carotenoids, primarily cantaxanthin which, particularly in salmonids, imparts a red colour to muscles making them look similar to those obtained from individuals living under natural conditions (Simpson et al., 1981; Choubert, 1983; Storebakken et al., 1986; Torrissen et al., 1989). Canthaxanthin, however, is not a vitamin A precursor and thus does not improve the nutritive quality of the fish meat.

Vitamin A is formed from those carotenoids only that have at least one β -ionic ring without hydroxy or other groups imparting xanthophyll character to carotenoids (Bauerfeind, 1972). In the materials studied, such carotenoids were β -carotene producing 2 vitamin A molecules and β -cryptoxanthin and hydroxyechinenone, yielding one vitamin A molecule each. Canthaxanthin belongs to ketocarotenoids which dye the tissues red. Muscles of *C. gariepinus*, as shown in the present study, contain about 25% keto-carotenoids. As demonstrated in earlier research (Czczuga and Dąbrowski, 1983; Czczuga and Kiziewicz, 1985), not all food carotenoids are absorbed by fish; carotenoid absorption is species-specific. *C. gariepinus* individuals had absorbed different food carotenoids, both those coloring the muscles (ketocarotenoids) and vitamin A precursors (β -carotene, β -cryptaxanthin, and hydroxyechinenone).

Table 4

Total carotenoid content in muscles of some freshwater fish species

Species	Carotenoid content ($\mu\text{g g}^{-1}$ w.w.)	Author
<i>Abramis brama</i> (L.)	0.013 – 0.128	Czczuga, 1982a
<i>Acerina cernua</i> (L.)	0.601	Czczuga, 1979b
<i>Coregonus albula</i> (L.)	0.162 – 0.420	Czczuga, 1977a
<i>Coregonus peled</i> (Gmel.)	0.002 – 0.170	Czczuga, 1977a
<i>Ctenopharyngodon idella</i> (Val.)	0.432	Czczuga, 1972
<i>Cyprinus carpio</i> L.	0.040 – 0.455	Czczuga, 1979a
<i>Hucho hucho</i> (L.)	0.505 – 0.788	Czczuga et al., 1986
<i>Misgurnus fossilis</i> (L.)	0.174 – 0.320	Czczuga, 1980
<i>Perca fluviatilis</i> L.	0.040	Czczuga, 1979b
<i>Salmo gairdneri</i> Rich.	0.018 – 0.212	Czczuga, 1979c
<i>Salmo trutta m. fario</i> L.	0.037 – 0.320	Czczuga, 1979c
<i>Salmo trutta m. lacustris</i> L.	0.256 – 0.777	Czczuga and Bartel, 1989
<i>Silurus glanis</i> L.	0.010	Czczuga, 1977b
<i>Thymallus thymallus</i> (L.)	0.198 – 0.200	Czczuga et al., 1985
<i>Tinca tinca</i> (L.)	0.145 – 0.320	Czczuga, 1992

Moreover, in spite of the young age of the fish examined, the total carotenoid content was rather high (Table 4), muscles being particularly carotenoid-rich, compared to other fish species. It should be remembered that carotenoid content in fish increases with age (Czeczuga, 1982b). The values found in the African catfish fry are generally close to those found in muscles of salmonids.

REFERENCES

- Babiker M.M.**, 1984: Aspect of the biology of the catfish *Clarias lazera* to its economic cultivation. *Hydrobiologia*, **110**: 295–304.
- Bauernfeind J.C.**, 1972: carotenoid vitamin A precursors and analogs in foods and feeds. *J. Agr. Food Chem.*, **20**: 456–473.
- Britz P.J., T. Hecht**, 1987: Temperature preferences and optimum temperature for growth of African sharp-tooth catfish (*Clarias gariepinus*) larvae and postlarvae. *Aquacult.*, **63**: 205–214.
- Choubert G.**, 1983: Effects d'un pigment caroténoïde, la canthaxanthine sur la pigmentation de la truite arc-en-ciel *Salmo gairdneri* Rich. *Bull. Franc. Piscic.*, **289**: 12–127.
- Czeczuga B.**, Carotenoids and vitamin A in phytophagous fish from heated waters, *Verh. Internat. Verein. Limnol.*, **18**: 1198–1203
- Czeczuga B.**, 1975: Carotenoids in fish. salmonidae and Thymallidae from polish water. *Hydrobiologia*, **46**: 223–239.
- Czeczuga B.**, 1977a: Carotenoids in fish. *Coregonus peled* (Gmel.) from polish water. *Acta Hydrobiol.*, **19**: 183–190.
- Czeczuga B.**, 1977b: Carotenoids in fish. *Silurus glanis* L.. *Pol. Arch Hydrobiol.*, **24**: 563–567.
- Czeczuga B.**, 1979a: Changes in carotenoids in *Cyprinus carpio* L. *Hydrobiologia*, **65**: 233–240.
- Czeczuga B.**, 1979b: Carotenoids in fish. Percidae from polish water. *Acta Hydrobiol.*, **21**: 1–7.
- Czeczuga B.**, 19779c: Carotenoids in *Salmo gairdneri* Rich. and *Salmo trutta morpha fario* L. *Hydrobiologia*, **64**: 251–259.
- Czeczuga B.**, 1980: Carotenoids in fish. Cobitidae from polish water. *Acta Hydrobiol.*, **22**: 147–155.
- Czeczuga B.**, 1982a: Carotenoids in fish. Cyprinidae: *Abramis brama*, *Abramis ballerus* and *Blicca björkna*. *Acta Hydrobiol.*, **24**: 275–281.
- Czeczuga B.**, 1982b: Carotenoids in uneven aged individuals of certain Antarctic species of fish. *Acta Ichthyol. Pisc.*, **12**, 1: 107–116.
- Czeczuga B.**, 1989: Studies on carotenoids in spawning *Salmo trutta morpha lacustris* L. *Acta Ichthyol. Pisc.*, **19**, 1: 49–58.
- Czeczuga B.**, 1992: Carotenoids in fish. Cyprinidae – Benthosophages: *Carassius carassius*, *Carassius auratus gibelio*, *Tinca tinca*, *Vimba vimba*, *Barbus barbus* and *Barbus meridionalis petenyi*. *Acta Ichthyol. Pisc.*, **22**, 2: 3–16.
- Czeczuga B., R. Czerpak**, 1968: carotenoids in the carapaces of the *Orconectes limosus* (Taf.), Crustacea, decapoda. *Eur. J. Biochem.*, **5**: 429–432.
- Czeczuga B., R. Czerpak**, 1976: The kind of food and the content of carotenoids and vitamin A in *Carassius carassius* (L.) and *Leucaspis delineatus* (Heck.). *Acta Hydrobiol.*, **18**: 1–21.
- Czeczuga B., K. Dąbrowski**, 1983: Carotenoids in diets and fish tissues. *Z. Tierphysiol. Tier-nähr. Futtermittelkde.*, **50**: 52–61.
- Czeczuga B., B. Kiziewicz**, 1985: Assimilation of rhodoxanthin from the food by fish. *Zool. Pol.*, **32**: 175–182.

- Czczuga B., A. Witkowski, M. Kowalewski**, 1985: Presence of salmoxanthin in *Thymallus thymallus* (L.) specimens. *Acta Ichthyol. Pisc.*, **15**, 1: 73–81.
- Czczuga B., A. Witkowski, M. Kowalewski**, 1986: carotenoids contents in *Hucho hucho* (L.) individuals. *Acta Ichthyol. Pisc.*, **16**, 1: 61–72.
- Czaerwiński J.**, 1984: Salinity tolerance of young catfish, *Clarias lazera* (Burchell). *J. Fish Biol.*, **25**: 147–149.
- Davies B.H.**, 1976: Carotenoids. In: Chemistry and biochemistry of plant pigments [T.W. Goodwin (ed.)], Academic Press, London–New York–San Francisco: 38–165.
- Richter C.J.J.**, 1976: The African catfish *Clarias lazera* (C. and V.), A new possibility for fish culture in tropical region. *Aspects of Fish Culture and Fish Breeding*, Misc. Pap., Agric. Univ., Wageningen, **13**: 51–71.
- Simpson K.L., T. Katayama, C.O. Chichester**, 1981: Carotenoids in fish feeds. In: carotenoids as Colorants and Vitamin A Precursors [J.C. Bauernfeind (ed.)], Academic Press, New York, Chapter 4: 463–538.
- Storebakken T., P. Foss, I. Huse, A. Wandsvik, T.B. Lea**, 1986: Carotenoids in diets for salmonids. III. Utilization of canthaxanthin from dry and wet diets by Atlantic salmon, rainbow trout and sea trout. *Aquacult.*, **51**: 246–255.
- Straub O.**, 1987: Key to carotenoids. Birkhauser Verlag, Basel–Boston.
- Torrissen O.J., R.W. Hardy, K.D. Schearer**, 1989: Pigmentation of Salmonids – carotenoid deposition and metabolism. *Critical Rev. – in Aquatic Sci.*, **1**: 209–225.
- Van der Waal B.C.W.**, 1974: Observations on the breeding habits of *Clarias gariepinus* (Burchell). *J. Fish Biol.*, **6**: 23–27.
- Viveen W.J.A.R., C.J.J. Richter, P.G.W.J. van Oordt, J.A.L. Janssen, G.A. Huisman**, 1985: Practical manual for the culture of the african catfish (*Clarias gariepinus*). Netherlands Minister for Development Cooperation, Section for Research and Technology. The Hague, The Netherlands.
- Viveen W.J.A.R., C.J.J. Richter, P.G.W.J. van Oordt, J.A.L. Janssen, G.A. Huisman**, 1985: Practical manual for the culture of the african catfish (*Clarias gariepinus*). Publication of: Directorate General International Cooperation of the Ministry of Foreign Affairs. The Hague, The Netherlands.

Translated by: Dr Teresa Radziejewska

Bazyli CZECHUGA, Bernard KŁYSZEJKO

ZASOBNOŚĆ W KAROTENOIDY NARYBKU SUMA AFRYKAŃSKIEGO (*CLARIAS GARIEPINUS* BURCHELL)

STRESZCZENIE

Autorzy stosując chromatografię kolumnową i cienkowarstwową badali występowanie i zawartość poszczególnych karotenoidów w skórze, mięśniach, jelitach i wątrobach 5-cio miesięcznego narybku suma afrykańskiego – *Clarias gariepinus*.

W wyniku badań ustalono obecność następujących karotenoidów: β -karoten, β -kryptoksantina, luteina, tunaksantyna, taraksantyna, anteroksantyna, hydroksyechinenon, kantaksantyna, α - β -doradeksantyna, astaksantyna, wiolaksantyna oraz mutatoksaantyna.

Podano również stosunki procentowe poszczególnych karotenoidów oraz ogólną ich zawartość, a także zawartość tych karotenoidów, które stanowią prowitaminę witaminy A.

Received: 1993.01.11

Author's address:

Prof. D.Sc. Bazyli Czczuga
Department of General Biology
Academy of Medicine in Białystok
Kilińskiego 1
15-230 Białystok

Prof. D.Sc. Bernard Kłyszajko
Department of Fish Physiology
University of Agriculture in Szczecin
Kazimierza Królewicza 4
71-550 Szczecin
Polska (Poland)