ACTA ICHTHYOLOGICA ET PISCATORIA

Vol. XXV, Fasc. 1 Szczecin 1995

Bazyli *CZECZUGA*

Carotenoids in fish

CAROTENOID IN YOUNG FORMS OF SOME STURGEONID FISH (*ACIPENSERIDAE*)*

KAROTENIODY U MŁODOCIANYCH FORM NIEKTÓRYCH RYB JESIOTROWATYCH (*ACIPENSERIDAE*)*

Department of General Biology, Academy of Medicine, Białystok, Poland

The occurrence and contents of various carotenoids in different body parts of young forms of three sturgeonid species were studied with column and thin layer chromatography.

INTRODUCTION

Up to the present, studies of carotenoids in fish have been limited mainly to the representatives of two families - Salmonidae [Torrissen et al. 1989], or Cyprinidae [Czeczuga 1992; 1993a; 1994], due to great economic importance they acquired as a result of a large-scale fish culture of certain species of these families.

In the last years sturgeonid fish have aroused greater interest in Poland [Kolman 1993], manifested in the spawn import, hatch of various sturgeon species and their hybrids, and also their-breeding.

Therefore, we made an attempt to establish the carotenoids composition in respective parts of sturgeonid fish in order to enrich the knowledge in this field, since carotenoids in sturgeonid representatives had been examined only of eggs in the *Acipenser ruthenus* L. [Czeczuga 1971], *Acipenser güldenstädti* Brandt [Czeczuga 1982] and to evaluate the amount of these carotenoids being a source of vitamin A.

* Part 53 in the series "Carotenoids in fish"

MATERIAL AND METHODS

Investigations were conducted on the following sturgeonid fish species: *Acipenser güldenstädti* Brandt, *Acipenser baeri* Brandt and bester, a hybrid of beluga (*Huso huso)* L. (♀) and sterlet (*Acipenser ruthenus* L. ♂), collected in the first decade of August 1993 in the A. Lityński Stock Centre of PAU in Gawrych Ruda, Suwałki district.

Each sample, when homogenised was flooded with 95 % acetone in a dark glass bottle and kept until analysed in refrigerator. Separation of particular carotenoids was done by the column and thin-layer chromatography, described in details in our previous paper [Czeczuga, Czerpak 1976]. Before, the material underwent hydrolysis with 10 % KOH in N_2 atmosphere, at room temperature, within 24 hours. When hydrolised an extract was transferred into column filled up with Al_2O_3. The length of column ranfed from 15 to 5 (Quickfit Co. - England). Particular fractions were eluted with various compositions of solvents [Czeczuga, Czerpak 1976].

Independently from column chromatography, the acetone extract was splitting up into particular fractions by thin-layer chromatography. For that glass plates covered with silicagel were used with different developing solvents [Czeczuga, Czerpak 1968]. Next R_f was counted according to the generally applied principles.

Identification of particular carotenoids was based on following methods: a/ appearance of the column chromatogrammes, b/ maximal carotenoids absorption in various solvents, c/ epiphese to hypophase relation determined in hexane and 95 % methanol, d/ comparison of the R_f values of thin-layer chromatogrammes; for identification of β-carotene, β-cryptoxanthin, canthaxanthin, lutein, zeaxanthin, doradexanthin, idoxanthin and astaxanthin - the chromatography standards of Hoffman-La Roche Co., Co. Ltd. Basel, Switzerland and Sigma Chemical Co. USA were applied; e/ presence of allylohydroxy groups identified with acidic chloroform, f/ epoxidic test. The quantity of particular carotenoids was estimated based upon a quantitive coefficients E 1 %/cm for adequate absorption maxima with ether or hexane [Davies 1976]. The chemical structure of particular carotenoids was presented according to Straub [1987].

RESULTS

In the material examined 16 carotenoids was found (Tab. 1, Fig. 1): most of them were in the bester individuals (15), fewest in the *Acipenser baeri* (11) and in the *Acipenser güldenstädti* 13 carotenoids were identified (Tab. 2)

α-carotene was found only in the *Acipenser baeri* individuals, and idoxanthin only in the bester. Dominating carotenoids in the *Acipenser güldenstädti* (Tab. 3) were: zeaxanthin (fins), doradexanthin (skin, liver and intestines) and tunaxanthin (muscles) and in the *Acipenser baeri* individuals: zeaxanthin (fins, muscles, liver and intestines) and doradexanthin (skin) (Table. 4). Doradexanthin dominated in all parts of the bester individuals (Tab. 5). The total carotenoid content was the highest in the intestines and liver of all the individuals examined. The total carotenoid content in the muscles varied slightly and ranged from 0.706 (*Acipenser baeri*) to 0.743 μg g^{-1} of fresh mass (*Acipenser güldenstädti*).

Table 1

List of the carotenoids from the investigated materials

Lp.	Carotenoid	Structure (see Fig. 1)	Semi-systematic name
1	ε - Carotene	A - r - A	ε, ε - carotene
2	α - Carotene	A - r - B	β, ε - carotene
3	β - Carotene	B - r -B	β, β - carotene
4	Neothxanthin	A - r - C	ε, ε - caroten-3-ol
5	β –Cryptoxanthin	B - r - D	β, β - caroten-3-ol
6	Tunaxanthin	C - r - C	ε, ε - carotene-3,3'-diol
7	Lutein	C - r - D	β, ε - carotene-3,3'-diol
8	Zeaxanthin	D - r - D	β, β - carotene-3,3'-diol
9	Canthaxanthin	E - r - E	β, β - carotene-4,4'-dione
10	Doradexanthin	C - r - F	3,3' - dihydroxy-β, β - carotene-4-one
11	Idoxanthin	F - r - G	3,3'-4' -Tihydroxy-β, β -caroten-4-one
12	Astaxanthin	F - r - F	3,3' - dihydroxy-β, β - carotene-4,4'-dione
13	Lutein epoxide	C - r - H	5,6 - epoxy-5,6,-dihydro-β, ε - carotene-3,3'-diol
14	Mutatoxanthin	D - r_1 - I	5,8–epoxy-5,8-dihydro-β, β-carotene-3,3'-diol
15	Rhodoxanthin	K -r_2 - K	4',5'- Didehydro-4,5'-retro-β, β-carotene-3,3'-dione
16	Bixin	L - r - M	methyl hydrogen 9'-cis-6,6'-diapocarotene-6,6'-dioate

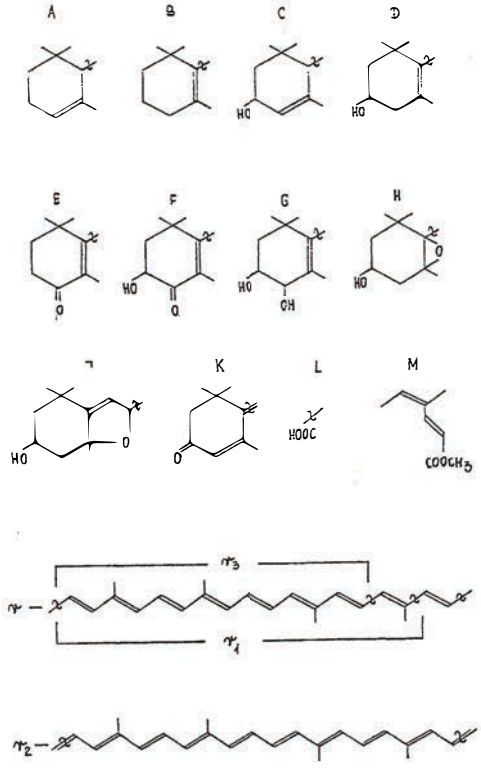

Fig. 1. Structural features of carotenoids from investigated materials

Table 2

Carotenoid content in the investigated fishes

Carotenoid	Sturgeonid		
	A.güldenstädti	*A.baeri*	Bester
ε - Carotene	x		x
α - Carotene		x	
β - Carotene	x	x	x
Neothxanthin	x	x	x
β –Cryptoxanthin	x	x	x
Tunaxanthin	x	x	x
Lutein	x	x	x
Zeaxanthin	x	x	x
Canthaxanthin	x	x	x
Doradexanthin	x	x	x
Idoxanthin			x
Astaxanthin	x		x
Lutein epoxide	x	x	x
Mutatoxanthin		x	x
Rhodoxanthin	x		x
Bixin	x		x
Number of carotenoid	13	11	15

Table 3

Carotenoid content in the *Acipenser güldenstädti* (l.t. 33.7 cm), in %

Carotenoid	Fins	Skin	Muscles	Liver	Intestine
ε - Carotene	3.3				
β - Carotene	2.1	1.1	5.4	1.5	1.7
Neothxanthin		3.9			
β –Cryptoxanthin		6.7	5.2		11.8
Tunaxanthin	6.1	14.2	27.5	4 .4	5.0
Lutein				10.3	
Zeaxanthin	32.9	20.7	6.2	26.2	32.8
Canthaxanthin				7.5	
Doradexanthin	18.2	22.7	23.0	38.5	42.2
Astaxanthin	20.3	16.9	19.8	4.0	
Lutein epoxide	15.6	11.1	10.3	7.6	6.5
Rhodoxanthin	1.5		2.6		
Bixin		2.7			
Total content in μg g^{-1} fresh wt	3.420	0.428	0.74 3	6.104	5.011

Table 4

Carotenoid content in the *Acipenser baeri* (l.t. 24.4cm), in %

Carotenoid	Fins	Skin	Muscles	Liver	Intestine
α - Carotene	-	-	-	1.0	-
β - Carotene	-	-	-	4.6	3.6
Neothxanthin	6.3	-	-	-	1.4
β –Cryptoxanthin	2.2	-	-	8.9	-
Tunaxanthin	10,3	10.1	14.3	6.7	6.8
Lutein	-	21.8	32.9	-	11.3
Zeaxanthin	45.3	27.8	39.0	27.0	40.1
Canthaxanthin	-	-	-	10.1	3.6
Doradexanthin	33.3	40.3	13.8	18.1	33.2
Lutein epoxide	2.6	-	-	-	-
Mutatoxanthin	-	-	-	23.6	-
Total content in μg g^{-1} fresh wt	6.676	0.640	0.706	17.626	25.671

Table 5

Carotenoid content in the bester (l.t. 24.0 cm), in %

Carotenoid	Fins	Skin	Muscles	Liver	Intestine
ε - Carotene	1.7	-	-	-	-
β - Carotene	3.0	6.3	12.9	3.7	5.6
Neothxanthin	7.3	-	5.9	3.8	-
β –Cryptoxanthin	5.8	12.0	0.5	5.2	-
Tunaxanthin	7.6	-	12.0	13.4	6.6
Lutein	9.2	6.7	-	9.5	9.7
Zeaxanthin	2.3	-	7.8	18.4	-
Canthaxanthin	-	-	-	-	7.5
Doradexanthin	31.1	47.0	16.9	34.1	33.8
Idoxanthin	-	-	-	-	7.5
Astaxanthin	24.0	9.9	-	5.8	26.7
Lutein epoxide	8.5	7.2	9.8	5.3	-
Mutatoxanthin	-	-	15.3	-	-
Rhodoxanthin	0.5	4.8	1.5	0.8	-
Bixin	-	6.1	17.4	-	2.6
Total content in μg g^{-1} fresh wt	10.949	1.144	0.731	15.518	17.461

DISCUSSION

Comparing carotenoid composition in the sturgeonid fish specimens examined (Tab. 2) it should be stated that 9 out of 16 carotenoids found were common for all the individuals. These are: β-carotene, neothxanthin, β-cryptoxanthin, tunaxanthin, lutein, zeaxanthin, canthaxanthin, doradexanthin and lutein epoxide. It should be noted that studies of carotenoids in eggs of *Acipenser ruthenus* L. [Czeczuga 1971] revealed the presence of β-carotene, tunaxanthin,

lutein, zeaxanthin and astaxanthin, and in eggs of *Acipenser güldenstädti:* β-carotene, β-cryptoxanthin, lutein, tunaxanthin, zeaxanthin and astaxanthin [Czeczuga 1982 were found.

Noteworthy is the presence of idoxanthin in the intestines of bester and in the skin of *Acipenser güldenstädti* and also the presence of bixin in the skin, muscles and intenstines of bester. Idoxanthin was first reported in salt water *Isopoda* representatives - *Idotea metallica* [Herring 1969], and in fish it was found in *Tinca tinca* individuals [Czeczuga 1992]. Bixin, however, has not been found in fish so far, so this is the first report in this field. As it is known [Straub 1987], it belongs to the group of diapocarotenoids and is produced as a result of oxidizing degradation of precursors, being the main carotenoid of *Bixa orellana* grains (*Bixaceae* - a tropical plant family) [Karrer and Jucker 1948]. Lately, we have met this carotenoid in thallus of several lichen species [Czeczuga 1993b]. Animals do not synthetize carotenoids *de novo*, but only take them to the organism with food: some of them undergo further oxidation in the organism. Food composition is of vital importance for the carotenoid content in fish [Simpson et al. 1981]. It should be considered that bixin is of food origin and in the sturgeonid fish specimens examined it did not undergo any further transformation. Similar phenomenon was observed in young *Cyprinus carpio* individuals, with regard to apocarotenoids [Czeczuga, Dąbrowski 1983] and in *Carassius carassius* individuals absorbing rhodoxanthin from food and accumulating it without any further transformation [Czeczuga, Kiziewicz 1985]. Similar phenomenon was also observed in representatives of salmonid fish, with regard to canthaxanthin [Storebakken et al. 1986].

Literature reports that young forms of sturgeon feed on ground plankton-benthonic organisms [Kokova et al. 1984; Timejko, Bondarenko 1988]. In the A. Lityński Stock Centre of PAU I could observe the individuals of the fish species examined plucking algae off subwater objects, in addition to artificial feeding.

As it is known, aquatic invertebrate organisms and fish selectively absorb and accumulate respective carotenoids both from vegetable food [Liaaen-Jensen 1989] and from animal food [Torrissen et al. 1989]. This could justify differences noted between the sturgeonid fish individuals examined, although all they were kept in the same environmental and food conditions.

The total carotenoid content in the sturgeonid fish individuals examined is high, compared with other fish species. In the muscles, especially, the carotenoid content is close to that found in salmonid fish [Czeczuga 1979; Czeczuga, Chełkowski 1984; Czeczuga, Bartel 1989].

ACKNOWLEDGEMENTS

Author is grateful thanks to dr Mieczysław Hus (Suwałki) his kind help in obtaining specimens of this fishes.

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Bazyli *CZECZUGA*

KAROTENOIDY U MŁODOCIANYCH FORM NIEKTÓRYCH RYB JESIOTROWATYCH (*ACIPENSERIDAE*).

STRESZCZENIE

Stosując chromatografie kolumnową i cienkowarstwową badano występowanie karotenoidów w płetwach, skórze, mięśniach, wątrobie i jelitach *Acipenser baeri, A.güldenstädti* oraz bestera (krzyżówki *Huso huso* (L.) ♀ ze *Acipenser ruthenus* L. ♂).

U osobników badanych gatunków ustalono obecność takich karotenoidów, jak: α-karoten, ε-karoten, β-karoten, neoksantyna, β-kryptoksantyna, tunaksantyna, luteina, zeaksantyna, kantaksantyna, doradeksantyna, idoksantyna, astaksantyna, epoksyd luteiny, mutatoksantyna, rodoksantyna oraz biksyna.

Najmniej karotenoidów stwierdzono w skórze *A.güldenstädti*, 0.428 μg, a najwięcej - w jelitach *A.baeri*, 25.671 μg g^{-1} świeżej masy.

Received: 1994.09.19

Authors address:
Prof. D.Sc. B.Czeczuga
Academy of Medicine
Kilińskiego 1
15-230 Białystok
Polska (Poland)