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CAROTENOID COMPOSITION IN THE MUSCLES OF SIBERIAN STURGEON (*ACIPENSER BAERII* BR.) AND STERLET (*ACIPENSER RUTHENUS* L.) JUVENILES FED FEED SUPPLEMENTED WITH VITATON

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ABSTRACT. Column chromatography (CC), thin layer chromatography (TLC), and high performance liquid chromatography (HPLC) were used to test the impact of Vitaton, which contains a natural form of β-carotene, on the content of carotenoids in the muscles of Siberian sturgeon, *Acipenser baerii* Br., and sterlet, *Acipenser ruthenus* L., reared in recirculating systems. It was confirmed that the overall carotenoid content, including provitamin A, was significantly higher ($P < 0.05$) in the experimental groups of both species that were fed trout granulate supplemented with Vitaton in comparison with the control group fed unsupplemented feed. A total of 21 carotenoids were identified in the material examined; of these echinenone, 3'-hydroxyechinenone, α-cryptoxanthin, calthaxanthin, deepoxy neoxanthin, and α-doradexanthin occurred only in specimens that had been fed the feed supplemented with Vitaton.

Key words: SIBERIAN STURGEON (*ACIPENSER BAERII*), STERLET (*ACIPENSER RUTHENUS*), MEAT QUALITY, CAROTENOIDS, VITATON

INRODUCTION

Formulated feed is applied widely in lieu of natural feed in the intense rearing of fish species for human consumption. Although this contributes substantially to the growth of cultivated fish, some of the consumption and dietetic qualities of the meat are lower. Among other factors, this refers to the content of carotenoids, which in addition to their biological function (Ostroumova 1998), contribute to the red color of muscles, especially

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in salmonid fishes (Tsushima et al. 1999). This, in turn, increases the commercial appeal and nutritional value of the fish. Initially, it was attempted to supplement feed with synthetic carotenoids from the so-called red group, which included astaxanthin. While this increased production costs significantly (Torrissen et al. 1989), the biological function of this synthetic carotenoid was substantially lower than that of the natural substance (Gabrielsen and Austreng 1998). This is also why the trend to supplement formulated feed with carriers of natural carotenoids is of increasing importance. Extracts are derived from sources rich in carotenoids, such as crustaceans (Choubert and Luquet 1983), the flower petals of some plants (Lee et al. 1978), fruits (Kamata et al. 1977), and seeds (Czeczuga and Dąbrowski 1983). Extracts are also obtained from the lower fungi as their cells harbor large quantities of the appropriate carotenoids. These include some species of yeast, especially *Rhodotorula sanneii* Lod., which primarily synthesizes torularhodin (Savolainen and Gyllenberg 1970), or *Phaffia rhodozyma* Andr., which mainly synthesizes the caroteniod astaxanthin (Fontana et al. 1996). Recently, the fungus *Blakslea trispora* Thaxter, which mainly synthesizes β-carotene, has been used to this end (Gamygin et al. 2004, Kolman et al. 2004).

Taking the preceding into consideration, the subject of the current investigation was to examine the degree to which the fry of two sturgeon species reared in recirculating systems deposit carotenoids obtained from formulated feed supplemented with a natural form of β-carotene.

MATERIALS AND METHODS

The investigation was performed on juveniles of Siberian sturgeon, *Acipenser baerii* Br., and sterlet, *Acipenser ruthenus* L., with initial mean body weights of 309.6 ± 12.3 and 237.7 ± 4.0 g, respectively. Experimental cultivation was conducted for 60 days in tanks that were part of a closed water recirculating system. The fish of each species were divided into two groups (experimental and control; in replicates of three). The fish were fed formulated trout feed (Aller, Poland) to which, in the final manufacturing phase, no fat was added. The feed was divided into two portions. Fat alone was added to the first, which was fed to the control group (Table 1). An equal amount of fat supplemented with Vitaton in a quantity of 0.7 g kg^{-1} feed was added to the second portion and was used to feed the experimental group (Table 1).

TABLE 1

Proximate composition of the control and experimental (supplemented with Vitaton) feed

Feed	Dry mass (%)	Total protein (%)	Raw fat (%)	Ash (%)
Control	90.60	45.32	13.02	6.27
Experimental	91.01	45.15	13.32	6.44

Vitaton has been in production in Ukraine for several years, and it contains approximately 8% β-carotene. It is a natural product that is derived from a biotechnological process in which corn is processed with the fungus *Blakslea trispora,* which is a natural synthesizer of carotenoids. In addition to β-carotene, Vitaton contains a variety of vitamins, free and bound amino acids, and other essential fatty acids (Gamygin et al. 2004).

During the final phase of the experiment, 10 specimens from each group were caught randomly. After sacrificing (Propiscin, IFI Olsztyn, Poland) and decapitating them, tissue samples were taken from the dorsal (I), side (II), and ventral (III) muscles, and these were preserved in acetone and subjected to tests for carotenoid content. The presence of individual carotenoids in the material examined was determined with column chromatography (CC), thin layer chromatography (TLC), and high performance liquid chromatography (HPLC). An appropriate sub-sample of tissue was homogenized, then hydrolyzed in a KOH solution with 10% methanol in a nitrogen atmosphere for 24 hours in darkness and at room temperature. The extract obtained was then transferred to a Quickfit Co. column filled with Al_2O_3 (CC) or to a glass slide covered with a silicon gel by Merck Co. (TLC). Next, the individual CC and TLC fractions were rinsed using various solvent combinations. The eluents obtained from the individual fractions were then dissolved in one of four solvents (petroleum benzene, hexane, acetone, ethanol) and readings of maximum absorption were taken in the ultraviolet and visible light spectra. The details of column and thin layer chromatography can be found in Czeczuga (1986).

Some carotenoids were assayed with the two-phase high performance liquid chromatography technique. In order to do this, an ion reagent by Shimadzu (Japan) was added to an appropriate volume of the extract being tested. A Shimadzu SCL-6B gradient programmer and a Rheodyne 7125 injector were applied when performing HPLC. A Shimadzu SPD-6A spectrophotometer was applied for the ultraviolet and visible wavelengths of the various carotenoids. The fluorescence of some pigments were also

tested with a Shimadzu RF-535 detector. A detailed description of high performance liquid chromatography can be found in the publication by Mantoura and Llewellyn (1983). The various carotenoids were identified by comparing them to standard component data such as: a) the overall appearance of column chromatography, b) spectra in ultraviolet and visible light, c) the application of epi- and hypophase in hexane and 95% ethanol, d) the R_f value in thin-layer chromatographs according to Kraus and Koch (1996), e) quantity or absence of allylic OH groups determined by testing with $CHCl_3$, f) the epoxide test, and g) spectral analysis (see Vetter et al. 1971).

The standard pigments used were carotenoids from the Hoffman-La Roche Company (Switzerland), the International Agency for ^{14}C Determinations (Denmark), and the Sigma Chemical Company, USA. Quantitative assays of carotenoids were based on spectroscopy in ultraviolet and visible light according to the Davies method (Czeczuga 1986). The structure of the individual carotenoids is presented according to Straub (1987) and Czeczuga (1988). Significant differences ($P < 0.05$) in carotenoid content in the tissues of the fish from the experimental and control groups were determined using the t-Student test.

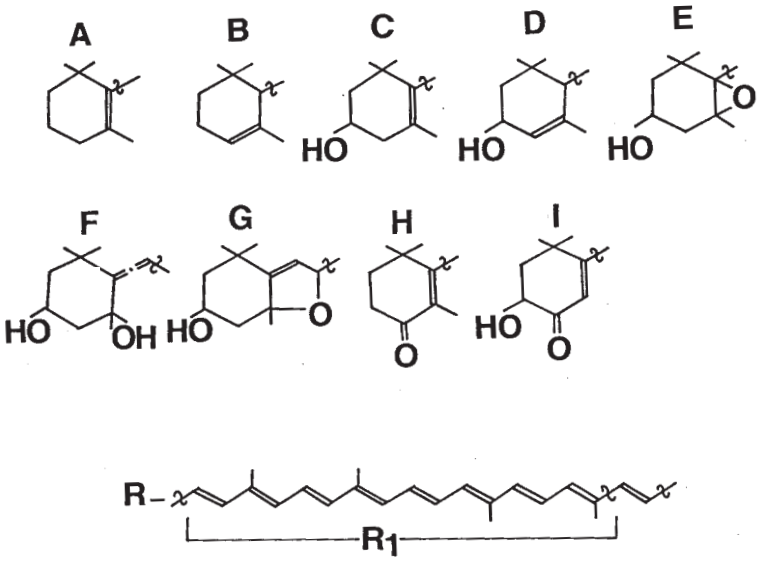

Fig. 1 Structural features of carotenoids from the investigated material: A-I, end group designation of carotenoids; R, R_1 – polyene chain (see Table 2).

RESULTS

A total of 21 carotenoids (Fig. 1, Table 2) were confirmed in the tested fry individuals, and these included four very rare carotenoids, namely, in Siberian sturgeon specimens – calthaxanthin and deepoxyneoxanthin, while in sterlet – α-cryptoxanthin and α-doradexanthin (Tables 3 and 4).

TABLE 2

List of carotenoids from the investigated material

No.	Carotenoid	Summary chemical Formula	Structure (see Fig. 1)	Semisystematic name
1.	α-carotene	$C_{40}H_{56}$	a-r-b	β,ε-Carotene
2.	β-carotene	$C_{40}H_{56}$	a-r-a	β,β-Carotene
3.	ε-carotene	$C_{40}H_{56}$	b-r-b	ε,ε-Carotene
4.	α-cryptoxanthin	$C_{40}H_{56}O$	b-r-c	β,ε-Carotene
5.	β-cryptoxanthin	$C_{40}H_{56}O$	a-r-c	β,β-Caroten-3-ol
6.	Neothxanthin	$C_{40}H_{56}O$	b-r-d	ε,ε -Caroten-3-ol
7.	Lutein	$C_{40}H_{56}O_2$	c-r-d	β,ε-Carotene-3,3'-diol
8.	Calthaxanthin	$C_{40}H_{56}O_2$	c-r-d	β,ε-Carotene-3,3'-diol (stereoisomeric)
9.	Zeaxanthin	$C_{40}H_{56}O_2$	c-r-c	β,β-Carotene-3,3"-diol
10.	Tunaxanthin	$C_{40}H_{56}O_2$	d-r-d	ε, ε-Carotene-3,3"-diol
11.	Lutein epoxide	$C_{40}H_{56}O_3$	d-r-e	5,6-Epoxy-5,6-dihydro-β,ε-carotene-3,3"-diol
12.	Antheraxanthin	$C_{40}H_{56}O_3$	c-r-e	5,6-Epoxy-5,6-dihydro-β,β-carotene-3,3"-diol
13.	Mutatoxanthin	$C_{40}H_{56}O_3$	c-r-g	5,8-Epoxy-5,8-dihydro-β,β-carotene-3,3"-diol
14.	Deepoxyneoxanthin	$C_{40}H_{56}O_3$	c-r1-f	6,7-Didehydro-5,6-dihydro-β,β-carotene-3,5,5-triol
15.	Echinenone	$C_{40}H_{54}O$	a-r-h	β,β-Caroten-4-one
16.	3'-hydroxyechinenone	$C_{40}H_{54}O_2$	c-r-h	3'-Hydroxy-β,β-caroten-4-one
17.	α-doradexanthin	$C_{40}H_{54}O_3$	d-r-i	3,3'-Dihydroxy-β,ε-caroten-4-one
18.	β-doradexanthin	$C_{40}H_{54}O_3$	c-r-i	3,3'-Dihydroxy-β,β-caroten-4-one
19.	Canthaxanthin	$C_{40}H_{52}O_2$	h-r-h	β,β-Carotene-4,4'-dione
20.	Phoenicoxanthin	$C_{40}H_{52}O_3$	h-r-i	3-Hydroxy-β,β-Caroten-4,4'-dione
21.	Astaxanthin	$C_{40}H_{52}O_4$	i-r-i	3,3'-Dihydroxy-β,β-Carotene-4,4'-dione

The dominant carotenoid in both of the species under investigation was the hydroxy carotenoid zeaxanthin and the ketocarotenoids β-doradexanthin and

TABLE 3

Carotenoid content in the muscles of Siberian sturgeon from experimental (Vitaton) and control groups (mean ± SD)

Muscles*	Carotenoid (see Table 1 and Fig. 1)	Major carotenoid (% of total carotenoids)	Total carotenoid content (μg g^{-1}w.w.) (n = 10)	Average I-III (μg g^{-1}w.w.)	β-carotene content (%)	Average I-III (%)	Content of provitamin A (%)	Average I-III (%)
			Vitaton					
I	1,2,4,5,6,7,8,9,10,12,14,15,16, 17,18,19,21	9 (40.6)	4.612 ± 0.011		12.4		19.8	
II	1,2,3,4,5,6,7,9,10,11,12,13,14, 15,16,17,19,20,21	21 (36.1)	5.076 ± 0.017	11.967[a] ± 1.382	10.1	12.0[a] ± 1.9	21.3	21.9[a] ± 2.0
III	1,2,3,4,5,7,8,9,10,12,14,15,16, 17,18,19,21	9 (29.4)	4.221 ± 0.009		13.4		24.5	
			Control					
I	1,2,3,5,7,9, 10,12,18,19, 21	18 (28.3)	2.021 ± 0.013		4.5		8.4	
II	2,3,5,7,9,10, 11,12,18,19,20,21	21 (31.6)	1.881 ± 0.008	4.636[b] ± 0.349	3.2	4.3[b] ± 0.8	6.8	8.8[b] ± 1.8
III	1,2,3,5,6,7,9,10,12,13,18,19, 20,21	9 (19.5)	1.945 ± 0.012		5.1		11.2	

**see Material and methods chapter; w.w. – wet weight; data in the same column with the same letter index do not differ significantly statistically (P > 0.05)*

TABLE 4

Carotenoid content in the muscles of sterlet from experimental (Vitaton) and control groups (mean ± SD)

Muscles	Carotenoid (see Table 1 and Fig. 1)	Major carotenoid (% of total carotenoids)	Total carotenoid content ($\mu g\ g^{-1}$ w.w.) (n = 10)	Average I-III ($\mu g\ g^{-1}$ w.w.)	β-carotene content (%)	Average I-III (%)	Content of provitamin A (%)	Average I-III (%)
			Vitaton					
I	1,2,3,4,5,6,7,8,9,10,12,14,15, 16,17,18,19,21	9 (32.8)	4.128 ± 0.016		10.8		21.9	
II	1,2,3,4,5,7,8,9,10, 11,14,15,16,17,18,19,21	21(36.1)	5.726 ± 0.012	5.404^{a} ± 0.938	15.3	14.7^{a} ± 3.0	18.4	22.8^{a} ± 4.1
III	1,2,3,4,5,6,7,8,9, 10,12,14,15,16,17,18,20,21	9(29.4)	6.358 ± 0.009		18.1		28.2	
			Control					
I	1,2,5,7,9,10,12,13,18,19,20, 21	18(32.4)	3.344 ± 0.007		4.6		9.4	
II	1,2,3,5,6,7,9,10,11,18,19,21	21(28.6)	2.551 ± 0.013	2.638^{b} ± 0.545	7.2	6.8^{b} ± 1.7	8.8	9.1^{b} ± 0.2
III	1,2,3,5,6,7,9,10,12,18,19,20, 21	18(31.2)	2.018 ± 0.015		8.6		9.2	

Denotations as in Table 3; data in the same column with the same letter index do not differ significantly statistically (P > 0.05)

astaxanthin. The overall carotenoid content, including that of provitamin A, was, on average, two to three-fold higher in all of the individuals of both species from the experimental groups fed the feed supplemented with Vitaton in comparison with the levels in the control groups (Tables 3 and 4). The differences that occurred between the carotenoid content in the sterlet was statistically significant ($P < 0.05$), while in the Siberian sturgeon they were highly statistically significant ($P < 0.01$). It was also verified that Vitaton supplementation has an impact on the variety of carotenoid content; only in the specimens fed the supplemented feed were echinenone and its derivative 3'-hydroxyechinenone and the other rare carotenoids mentioned previously confirmed.

DISCUSSION

Vitaton has been in production in Ukraine for several years (Gamygin et al. 2004). It contains approximately 8% natural β-carotene that is derived from a process in which corn kernels are transformed by the fungus *Blakslea trispora*, which is a representative of the class of Phycomycetes. The only carotenoid biosynthesis trail in this fungus begins with phytoene and ends with β-carotene. Initially, a series of acyclic carotenoids are formed which are then transformed into monocyclic γ-carotene, and this carries bicyclic β-carotene. This, in turn, accumulates in relatively significant quantities in the walls, cytoplasm, and mitochondria of the cells of this fungus (Goodwin 1980).

As the current study of feed supplemented with Vitaton exemplifies, the juvenile of both acipenserid species resulted in a statistically higher increases in overall carotenoid contents, a greater variety of carotenoids, a higher content of provitamin A carotenoids. An increase in the overall carotenoid content meant that the juvenile stages of the studied acipenserid species are more viable since these compounds have a positive impact on a range of life processes in fish (Bendich 1993, Ostroumova 1998).

The higher carotenoid content in both juvenile (Mikulin and Soin 1975) and adult stages of fish provides increased resistance to viral, bacterial, and fungal infections (Chew 1993), which can cause significant losses among acipenserid fry (Kokova et al. 1984). Carotenoids, especially β-carotene and astaxanthin, retard the development of cancer (Mathews-Roth 1985); additionally, carotenoids are antioxidants (Bendich 1993, Kabayashi and Sakamoto 1999).

Of the 21 carotenoids confirmed in the studied acipenserid juveniles, the majority had previously been identified in these species (Czeczuga 1971, 1982, 1995). The new carotenoids in acipenserid species were α-cryptoxanthin, calthaxanthin, deepoxyneoxanthin, and α-doradexanthin. These carotenoids are also not frequently found in fish, and α-cryptoxanthin is usually found in spermatophytes (Goodwin 1980) and was described for the first time in macrophytes by Weedon (1971). With regard to calthaxanthin, it was isolated for the first time in the flower petals of cowslip, *Caltha palustris* L., and some researchers refer to it as 3'-epilutein, as it is a stereoisomer of lutein (Goodwin 1980, Straub 1987). Deepoxyneoxanthin, as a derivative of neoxanthin, was first isolated in the flowers of *Trollius europeus* L. as trollein, while a similar carotenoid referred to as deepoxyneoxanthin was isolated simultaneously from the flowers of another plant *Mimulus guttatus* L. (Weedon 1971). Later investigations indicated that this is the same carotenoid that belongs to the group of polyhydroxy compounds (Straub 1987). As far as animals are concerned, deepoxyneoxanthin has been identified to date in salmonid fish (Torrissen et al. 1989) and in Nile tilapia, *Oreochromis niloticus* L. (Czeczuga et al. 2005). The last of the group of rare carotenoids is α-doradexanthin, which, as a derivative of α-carotene, was isolated for the first time in the muscles of gold fish (Katayama et al. 1970). At the end of the 1970's, another carotenoid was isolated in the cells of the green algae *Fritschella tuberosa* Braun, and its name, fritschiellaxanthin, was taken from the Latin name of the algae (Goodwin 1980). As it has been established, in this instance it is the same carotenoid that belongs to the group of ketocarotenoids (Straub 1987). Despite this, some botanists maintain that the name of this ketocarotenoid is fritschiellaxanthin, while zoologists – α-doradexanthin. These rare carotenoids as well as echinenone and its derivative 3'-hydroxyechinenone were only isolated in the sturgeon juveniles fed the feed supplemented with Vitaton.

As is known, some carotenoids are provitamin A. This refers to those carotenoids in which at least one of the of the two β-ion rings is free. Of the 21 carotenoids confirmed in the material investigated in the present study, those which belong to this group are α-carotene, β-carotene, β-cryptoxanthin, and echinenone. Of these carotenoids, the most abundant in provitamin A is β-carotene, from whose single molecule two molecules of vitamin A are formed, while from the others only one molecule is formed.

It should be emphasized that the fry of the two sturgeon species studied that were fed the feed supplemented with Vitaton contained substantially more β-carotene and the other provitamin A carotenoids. Investigations conducted in the second half of the twentieth century indicated that vitamin A in fish can be created both through reductive metabolic pathways from other carotenoids belonging to the hydroxy group or the ketocarotenoids. As far as the hydroxy carotenoids are concerned, those such as lutein, tunaxanthin, and zeaxanthin (yellow carotenoids) are transformed into all three forms of vitamin A (vitamins A_1 and A_2 alcohol, A_1 and A_2 aldehyde, and anhydro vitamins A_1 and A_2). This has been demonstrated in specimens of Nile tilapia (Katsuyama and Matsuno 1979). The literature regarding the transformation of ketocarotenoids into vitamin A in fish is far richer. Above all, this refers to the common fish carotenoids such as canthaxanthin and astaxanthin (red carotenoids). The transformation of them into vitamin A has been confirmed in specimens from such fish species as platy, *Xiphophorus variatus* Guent., guppy, *Poecilia reticulata* Peters (Gross and Budowski 1966), in specimens of mosquitofish, *Gambusia holbrooki* Grd. (Granguard 1962), in Nile tilapia (Matsuno et al. 1985), as well as in wild and cultivated specimens of rainbow trout, *Oncorhynchus mykiss* Walb. (Guillou et al. 1989). Both the hydroxy carotenoids and the ketocartenoids mentioned belong to the most common carotenoids not only among fish but also among aquatic animals on the whole. In the eggs of acipenserids (Czeczuga 1971, 1982), in fish for trade (Czeczuga 1995), as well as in the currently studied fish, these carotenoids comprise a significant portion of all the carotenoids. It is also most likely that the source of vitamin A in acipenserids is the ketocarotenoids and hydroxy carotenoids.

It must be emphasized that feeding fish feed supplemented with Vitaton does not only influence an increase in the carotenoid content and their wider variety but also influences increased fat accumulation in their muscles. This refers not only to acipenserid fish (Gamygin et al. 2004), but also to rainbow trout (Kolman et al. 2004). Increased fat content with a high proportion of polyunsaturated fatty acids as well as carotenoids contributes substantially to improving the dietary qualities in the meat of a given species.

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STRESZCZENIE

BADANIA NAD DEPONOWANIEM KAROTENOIDÓW Z PASZY W CIELE NARYBKU JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERII* BR.) I STERLETA (*ACIPENSER RUTHENUS* L.)

Autorzy stosując chromatografię kolumnową (CC), cienkowarstwową (TLC) oraz wysokosprawną chromatografię cieczową (HPLC) badali wpływ preparatu Vitaton zawierającego naturalną formę β-karotenu na zawartość karotenoidów w narybku jesiotra syberyjskiego, *Acipenser baerii* Br. i sterleta, *Acipenser ruthenus* L. podchowywanego w obiegach recyrkulacyjnych. Ustalono, że u narybku tych dwóch gatunków jesiotrów karmionych pstrągową paszą granulowaną firmy Aller-Pl z dodatkiem preparatu Vitatonu była znacznie większa ogólna zawartość karotenoidów oraz tych, które stanowią prowitaminę witaminy A (α-, β-karotenu, β-kryptoksantyny i echinenonu) ($P < 0.05$; tab. 3 i 4). W badanym materiale ustalono obecność 21 karotenoidów (rys. 1, tab. 1) wśród których takie jak echinenon, 3'-hydroksychinenon, α-kryptoksantyna, kaltaksantyna, diepoksyneoksantyna i α-doradeksantyna wystąpiły tylko u osobników obu gatunków odżywianych paszą z preparatem Vitaton. Dominującymi karotenoidami u osobników obu gatunków okazały się zeaksantyna, β-doradeksantyna oraz astaksantyna.