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Fish carotenoids

CAROTENOIDS IN THE COMMON- AND GOLDEN FORM OF RAINBOW TROUT, *ONCORHYNCHUS MYKISS* WALBAUM

KAROTENOIDY U OSOBNIKÓW ZWYKŁEJ I ZŁOCISTEJ FORMY PSTRĄGA TĘCZOWEGO, *ONCORHYNCHUS MYKISS* WALBAUM

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The authors investigated carotenoids in several body parts of the common- and golden form of the rainbow trout.

INTRODUCTION

The aquaculture of salmonids, carried out on a large scale, provides fish meat which is more pale than the meat of wild individuals, and thus less economically attractive (Czeczuga 1975; Czeczuga et al. 1991; Bjerkeng et al. 1992). Therefore, carotenoid-rich material or carotenoids themselves have been added to fish feed to make the meat of farm fish resemble in colour the meat of wild fish growing in natural conditions. Various components of carotenoid-rich organisms have been added to fish feed, including flower petal extracts of *Tagetes erecta*, *Curcubita marcia*, or *Hyppophae rhamnoides* (Lee et al. 1978; Kamata et al. 1977) and *Aesculus* sp. (Neamtu et al. 1976), dried algae of the genus *Spirulina* sp. (Choubert 1979), and yeasts *Rhodotorula sanneii* (Savolainen and Gyllenberg 1970), and *Phaffia rhodozyna* (Johnson et al. 1977). Animal species used as a feed additive include krill meat (Lambertsen and Braekkan 1971) or oil (Lambertsen and Braekkan 1971; Fujita et al. 1983; Arai et al. 1987), plankton crustacean—*Calanus finmarchicus*, found in great numbers in oceans (Lambertsen and Braekkan 1971), numerous shrimps (Saito and Regier 1971; Choubert and Luquet 1983), crabs (Spinelli et al. 1974; Kuo et al. 1976), and even fish oil from *Mallotus villosus* (Lambertsen and Braekkan 1971).

Colour "improvement" of cultured salmonid meat is also achieved by the addition of certain carotenoids to the fish feed. Only in 1986 over 6 tons and approximately 15 tons of carotenoid pigments in 1990 were used on salmonid farms (Torrissen et al. 1989). Apoca-

rotenales (Hirao et al. 1962), and particularly ketocarotenoids such as canthaxanthin (Choubert and Luquet 1975, 1982; Choubert 1983; Foss et al. 1984; Storebakken et al. 1986) and astaxanthin (Torrissen and Braekkan 1979; Craik and Harvey 1986, 1987; Storebakken et al. 1987) have been used. The supplementation of fish feed with carotenoids raises the cost of fish production on the farm. It has been estimated that the increase reaches 10–15% (Hardy et al. 1990). This problem also occurs in Poland (Kuźmiński and Dobosz 1995).

In 1965 rainbow trout fry was imported to Poland from France (Maliszewski 1987). The fry included also the so called golden forms, characterised by orange colour.

Thus, we decided to analyse the carotenoid content in respective parts of these two forms of *Oncorhynchus mykiss* receiving the same feed.

MATERIAL AND METHODS

Matured individuals of *Oncorhynchus mykiss* Walbaum (syn. *Salmo gairdneri* Rich.) representing the common- and the golden form (5 specimens of each form; total length: 24.5–35.0 cm; weight: 190.5–207.5 g) were collected in summer from the Gawrych Ruda Fish Farm. Investigated fish were kept in the same pond and were receiving the same feed (pellet feed Dana, minced common bream, white bream and roach as 1:1). The following organs of both form were studied: fins, skin, muscles, liver, and intestine.

The material was prepared immediately on the collection site by placing it in dark glass containers and filled with 95% acetone. It was kept in a refrigerator until the spectrophotometric determinations were made.

The carotenoid pigments were extracted by means of 96% acetone in a dark room. Saponification was carried out by means of 10% KOH in ethanol at a temperature of about 20°C for 24 hours in the dark in a nitrogen atmosphere.

Columnar and thin-layer chromatography, described in detail in our previous paper (Czeczuga 1981) were used for the separation of the various carotenoids. A glass column (Quickfit, England), approx. 1 cm in diameter and 15–20 cm in length, filled with Al_2O_3, was used in column chromatography. The extract was passed through the column after which the different fractions were eluted with the solvent. Silica gel was used for the thin-layer chromatography, with the appropriate solvent systems, the R_f values being determined for each spot.

The pigments were identified by the following methods: (a) behaviour on column chromatography; (b) absorption spectra of the pigments in various solvents; (c) the partition characteristics of the carotenoid between hexane and 95% methanol; (d) comparison of R_f on thin-layer chromatography (for identification of α-, β-, ε-carotene, canthaxanthin, lutein, zeaxanthin, astaxanthin, and tunaxanthin the process of co-chromatography was applied

using identical carotenoids—Hoffman-La Roche and Co. Ltd, Basel Switzerland, and Sigma Chemical Company, U.S.A.); (e) the presence of allylic hydroxyl groups was determined with acid chloroform; (f) the epoxide test; and the mass spectrum of end groups.

Quantitative determinations of the concentrations of carotenoid solutions were from the quantitative absorption spectra. These determinations were based of the absorption coefficient E 1%/cm at the wave lengths of maximal absorbance in petroleum ether or hexane.

RESULTS

Fifteen carotenoids, derivatives of α-, β- and ε-carotene, were found in the two forms of *Oncorhynchus mykiss* (Tab. 1, Fig. 1). Worth noting is the finding of neothxanthin, idoxanthin, and deepoxyneoxanthin. Golden individuals contained a wider variety of carotenoids; only they contained β-cryptoxanthin, lutein, zeaxanthin and deepoxyneoxanthin. β-doradexanthin was found in common individuals. In both forms, astaxanthin was predominant in most body parts examined, while canthaxanthin in some (Tabs. 2, 3). All body parts of the golden form were more abundant in carotenoids, compared with the common form. Moreover, in the common individuals, the intestine and the liver were the most abundant in carotenoids, while in the golden form—the intestines and the fins. The muscles of the golden form were approximately five times richer in carotenoids.

Table 1

List of the carotenoids from the investigated material

No.	Carotenoid	Summary formula	Structure (see Fig. 1)	Semisystematic name
1	α-Carotene	$C_{40}H_{56}$	A - r - B	β,ε-Carotene
2	β-Carotene	$C_{40}H_{56}$	B - r - B	β,β-Carotene
3	ε-Carotene	$C_{40}H_{56}$	A - r - A	ε,ε-Carotene
4	β-Cryptoxanthin	$C_{40}H_{56}O$	B - r - C	β,β-Caroten-3-ol
5	Neothxanthin	$C_{40}H_{56}O$	A - r - D	ε,ε-Caroten-3'-ol
6	Lutein	$C_{40}H_{56}O_2$	C - r - D	β,ε-Carotene-3,3'-diol (stereoisomeric)
7	Zeaxanthin	$C_{40}H_{56}O_2$	C - r - C	β,β-Carotene-3,3'-diol
8	Tunaxanthin	$C_{40}H_{56}O_2$	D - r - D	ε,ε-Carotene-3,3'-diol
9	Antheraxanthin	$C_{40}H_{56}O_3$	C - r - E	5,6-Epoxy-5,6-dihydro-β,β,-carotene-3,3'-diol
10	Lutein epoxide	$C_{40}H_{56}O_3$	D - r - E	5,6-Epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol
11	Doradexanthin	$C_{40}H_{54}O_3$	C - r - G	3,3'-Dihydroxy-β,β-caroten-4-one
12	Canthaxanthin	$C_{40}H_{52}O_2$	F - r - F	β,β-carotene-4,4'-dione
13	Idoxanthin	$C_{40}H_{54}O_4$	G - r - H	3,3',4'-Triohydroxy-β,β-caroten-4-one
14	Astaxanthin	$C_{40}H_{52}O_4$	G - r - G	3,3'-Dihydroxy-β,β-carotene-4,4'dione
15	Deepoxynoexanthin	$C_{40}H_{56}O_3$	C - r - I	6,7-Didehydro-5,6-dihydro-β,β-carotene-3,5,3'-triol

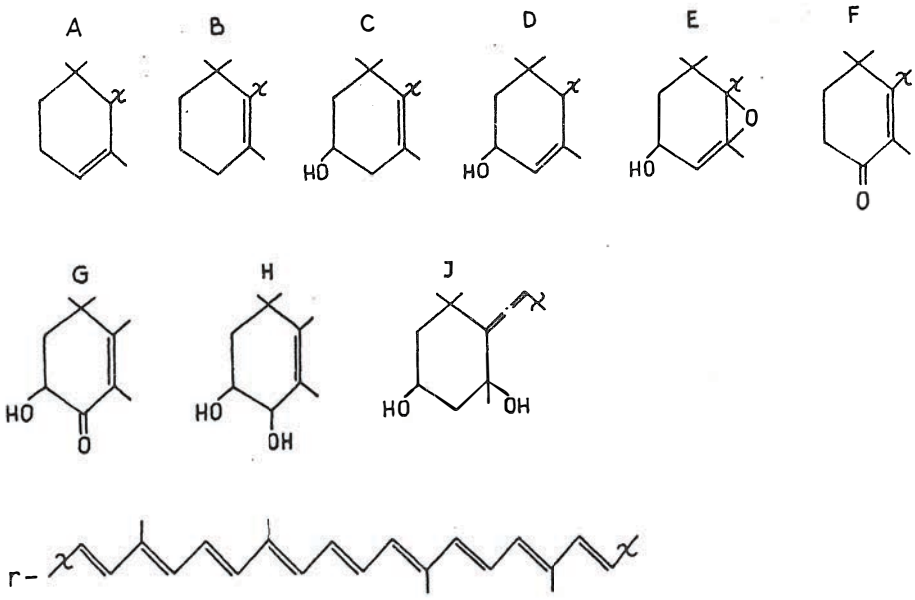

Fig. 1. Structural features of carotenoids from investigated materials (A–I end group designation carotenoids; r – polyene chain)

Table 2

Carotenoid content in selected body parts of the common form of the rainbow trout

Body parts	Carotenoids (see Table 1)	Major carotenoids (%)	Total content ($\mu g \cdot g^{-1}$ fresh weight)
Fins	8, 9, 12, 14	12 (46.9)	2.314
Skin	2, 9, 10, 12, 13, 14	14 (36.8)	0.727
Muscles	3, 8, 9, 10, 12, 13, 14	14 (34.0)	0.068
Liver	1, 2, 8, 10, 12, 14	14 (55.7)	3.683
Intestine	2, 5, 10, 11, 12, 14	14 (41.2)	5.806

Table 3

Carotenoid content in selected body parts of the golden form of the rainbow trout

Body parts	Carotenoids (see Table 1)	Major carotenoids (%)	Total content ($\mu g \cdot g^{-1}$ fresh weight)
Fins	4, 5, 6, 7, 8, 9, 12, 14	14 (27.1)	6.501
Skin	8, 12, 14, 15	12 (54.9)	1.027
Muscles	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15	14 (39.6)	0.339
Liver	2, 5, 7, 9, 12, 13, 14	14 (31.5)	5.373
Intestine	4, 5, 7, 12, 14	12 (37.2)	6.681

DISCUSSION

Most of the carotenoids found in both forms of the *Oncorhynchus mykiss* are common carotenoids of salmonid fishes (Torrissen et al. 1989). However, ε-carotene and its two derivatives—neothxanthin and tunaxanthin, as well as β-carotene derivatives—antheraxanthin, idoxanthin, and deepoxyneoxanthin have been rarely found in salmonids. In fishes ε-carotene was first described in the representatives of a marine fish—*Apodichthys flavidus* (c.f. Crozier and Wilkie 1966). In salmonid fishes we observed its presence in the fins of *Coregonus albula* (c.f Czeczuga 1977), in the fins and skin of wild individuals of *Oncorhynchus mykiss* and in the eggs of wild individuals of *Salmo trutta* morpha *fario* (Czeczuga 1979b). Moreover, ε-carotene was noted in the fins of *Hucho hucho* (c.f. Czeczuga et al.1986). Neothxanthin was first isolated from a marine fish *Neothunnus albacora* (Tanaka et al. 1977). In salmonids, we found this carotenoid in *Salmo trutta* morpha *lacustris* (c.f. Czeczuga and Bartel 1989) and *Salmo trutta* morpha *trutta* (c.f. Czeczuga and Chełkowski 1984), in both cases during spawning migrations. The other derivative of ε-carotene—tunaxanthin, first isolated from a tuna *Thunnus orientalis* (Hirao et al. 1957), forms at least three stereochemical variations (Rønneberg et al. 1978; Bingham et al. 1979; Matsuno et al. 1980b). Its occurrence in fish was described by Bingham et al. (1979). In salmonids, tunaxanthin was identified in freshwater species *Coregonus albula* and *Coregonus peled* (Czeczuga 1977; Dąbrowski et al. 1987) and in some anadromic species of Pacific salmon of the genus *Oncorhynchus* (Czeczuga 1979a; Matsuno et al. 1980b; Miki et al. 1982; Kitahara 1984a, b). Antheraxanthin as a derivative of zeaxanthin is common in plants. In salmonids, antheraxanthin was encountered in different body parts of several species of Pacific salmon of the genus *Oncorhynchus* (Matsuno et al. 1980a; Kitahara 1983, 1984a, b, 1985) and in male *Salmo trutta* morpha *lacustris* during their spawning (Czeczuga and Bartel 1989). Idoxanthin was first isolated from a sea crustacean *Idothea metallica* (c.f. Herring 1969). Its occurrence in fishes was reported by Nagata and Matsuno (1979) in *Cyprinus carpio* and by Miki et al. (1982, 1984) in the ovaries of several marine fish species. We found this carotenoid in the gonads of *Salmo trutta* morpha *trutta* (c.f. Czeczuga and Chełkowski 1984). Deepoxyneoxanthin was first isolated from the cells of *Euglena gracilis* (c.f. Nitsche 1974). Its occurrence in salmonids was reported by Schiedt et al. (1985) and Torrissen et al. (1989).

Investigations of radioactively marked carotenoids added to the feed given to *Oncorhynchus mykiss* (Hata and Hata 1973; Choubert et al. 1987; Hardy et al. 1990) have revealed their transformations. After 48 hours, β-carotene administrated to a rainbow trout is retained mostly in the alimentary tract, lutein in the alimentary tract and the skin, while zeaxanthin in the skin (Hata and Hata 1978). Moreover, marked canthaxanthin introduced

with fish feed has been found to be present in all body parts of fish as long as after 96 hours; approximately 40% is excreted with faeces to the water, while 10% is retained in the alimentary tract and 7% in the remaining body parts (Hardy et al. 1990). Similar results were obtained earlier by Choubert et al. (1987). Further transformations of canthaxanthin occur in the trout liver, resulting in the formation of bile which is then excreted. The activity of radioactive bile is eight times higher than of serum (Hardy et al. 1990). This suggests that considerable part of canthaxanthin introduced with feed is subject to transformations in the liver, while the blood carries slight amounts of canthaxanthin around the body.

The retention of particular carotenoids in the respective body parts is selective. The studies have revealed that the same carotenoid in individuals of one species is retained in the organism, while in other species—excreted. This refers both to plant-food carotenoids (Katsuyama and Matsuno 1979; Matsuno and Katsuyama 1979; Czeczuga 1981; Czeczuga and Kiziewicz 1985) and animal-food carotenoids (Hata and Hata 1975; Spinelli and Mahnken 1978; Miki et al. 1984). The studies of radioactively marked β-carotene and astaxanthin have demonstrated that the former not always becomes transformed into other xanthophylles, while the major part of the latter is absorbed by tissues of the respective body parts (Katayama et al. 1972a, b, 1974; Al-Khalifa and Simpson 1988). Frequently, the absorption of astaxanthin and canthaxanthin in salmonids is twenty times higher than of lutein and zeaxanthin (Schiedt et al. 1985). A wider variety of carotenoids in the golden form of the rainbow trout compared with the common form, kept in the same pond and fed the same diet could be explained by higher ability of golden form to retain carotenoids.

Taking into account extra costs of feed supplementation with carotenoids, which farmers of salmonid fishes, including *Oncorhynchus mykiss*, have to bear, the culture of golden individuals seems more profitable. Growing as quickly as the common form individuals, they have more attractive appearance of meat, which abounds in valuable carotenoids, some being vitamin A provitamin.

CONCLUSION

The present study was aimed at investigating the presence of various carotenoids in selected body parts of the common- and golden form of rainbow trout, *Oncorhynchus mykiss* Walbaum, kept in the same pond and receiving the same feed.

Fifteen carotenoids were found in the two forms of *Oncorhynchus mykiss*. Astaxanthin and canthaxanthin was the main component in all investigated parts of both forms of the rainbow trout.

The golden individuals contained a wider variety of carotenoids and all their body parts were more abundant in carotenoids then those of the common form. The muscles of

the golden form were approximately five times richer in carotenoids then those of the common form.

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Karotenoidy u osobników zwykłej i złocistej formy pstrąga tęczowego
Oncorhynchus mykiss Walbaum

STRESZCZENIE

Stosując chromatografię kolumnową i cienkowarstwową analizowano występowanie poszczególnych karotenoidów w mięśniach, płetwach, skórze, wątrobie i jelitach osobników zwykłej i złocistej formy pstrąga tęczowego, *Oncorchynchus mykiss* hodowanych w tym samym basenie i karmionych tą samą paszą.

U osobników obu form stwierdzono występowanie 15 karotenoidów, wśród których astaksantyna i kantaksantyna były głównymi karotenoidami występującymi w poszczególnych częściach ciała ryb.

Wszystkie badane części ciała osobników złocistej formy pstrąga tęczowego charakteryzowały się większą ogólną zawartością karotenoidów niż zwykłej formy, a mięśnie oprócz większej różnorodności karotenoidów wykazały duży (nawet pięciokrotny) wzrost ich koncentracji.

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