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CAROTENOID CONTENT IN THE BODY OF WHITEFISH (*COREGONUS LAVARETUS LAVARETUS* (L.)) FROM LAKE ŁEBSKO (NORTHERN POLAND)

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ABSTRACT. The aim of this study was to determine the impact of sex on the content of β-carotene, lutein, astaxanthin, canthaxanthin, zeaxanthin, and total carotenoids in the skin, muscles, and livers of whitefish (*Coregonus lavaretus lavaretus* (L.)). Fish samples for assays were collected from Lake Łebsko. There is a whitefish conservation program in Lake Łebsko. The results show that males are more carotenoid rich in comparison to females. The total carotenoids ranged from 0.39 μg g^{-1} wet weight (ww) (skin of females) to 1.55 μg g^{-1} ww (livers of males). The highest carotenoid levels were in whitefish livers (1.36-1.55 μg g^{-1} ww), and the lowest were in its skin (0.39-0.41 μg g^{-1} ww). Astaxanthin was the dominant carotenoid in whitefish muscles and livers. The highest levels of β-carotene were recorded in the skin of both whitefish males and females.

Key words: WHITEFISH (*COREGONUS LAVARETUS LAVARETUS*), CAROTENOIDS, LAKE ŁEBSKO

INTRODUCTION

Carotenoids are among the nutrients which must be supplied to fish with feed, and once ingested they are absorbed mainly in the median and terminal part of the intestine (Torrissen et al. 1990, Choubert et al. 1991) and then transported by lipoproteins (Ando et al. 1985, Nakamura et al. 1985). The absorption and distribution of carotenoids in fish is influenced not only by species but also by age and physiological state, feed type, and the environment inhabited by the fish (Foss et al. 1984, Ando 1986, Czeczuga et al. 1991, Bjerkeng et al. 1992, Storebakken and No 1992, Woźniak 2000). Carotenoids perform several metabolic functions in fish. Most importantly, they are a precursor of vitamin A (Al-Khalifa and Simpson 1988). Another function of carotenoids that

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has been well investigated is their effect on the color of the skin, muscles, and fins of salmonids and other fish species (Boonyaratpalin and Unprasert 1989). They also play an important role in fish reproduction as they have a beneficial effect on spawning performance, depress larval mortality, and enhance growth rate (Dąbrowski et al. 1987, Christiansen et al. 1994, Christiansen and Torrissen 1996, George et al. 2001). Karnaukhov et al. (1977) suggests that xanthophylls perform certain respiratory functions which are activated under low oxygen levels in the environment (Palloza and Krinsky 1992, Krinsky 1993, Tsuchihashi et al. 1995). It was also discovered that the presence of these compounds in feed ingested by fish contributes to an increased number of immune bodies in them (Thompson et al. 1995).

Studies of the roles of carotenoids and their concentrations in fish have focused primarily on salmonids. It is worth noting that, although carotenoids play various functions and occur in various concentrations, many fish species have yet to be analyzed for the presence of these compounds. Data is also scarce regarding the functions of carotenoids in the metabolism of coregonoids (Czeczuga 1975, Dąbrowski et al. 1987, Czeczuga et al. 1991).

The aim of the present study was to determine the total content of carotenoids in the skin, muscles, and livers of whitefish, *Coregonus lavaretus lavaretus* (L.), males and females from Lake Łebsko and the percentage of particular carotenoids in these tissues.

MATERIAL AND METHODS

Whitefish specimens were collected from Lake Łebsko located in Słowiński National Park (northern Poland). A sample of 21 fish was netted in the 2000-2001 period. The body weight of the fish ranged from 500 to 1420 g and the body length was from 32.5 to 44.7 cm.

The total carotenoids concentration and the content of β-carotene, lutein, astaxanthin, canthaxanthin, and zeaxanthin were analyzed in the skin, muscles, and livers of the whitefish.

Each tissue sample was homogenized, mixed with 95% acetone and extracted for 48 h in the dark at 4ºC. Afterwards, the samples were hydrolyzed with 5% KOH for 24 hours at room temperature. Next, the samples were neutralized with acetic acid to pH 7 and re-extracted in benzene and petroleum ether. The re-extracted samples were transferred into a chromatographic column filled with aluminum oxide in which individual

carotenoids were eluted by the method described by Czeczuga and Czerpak (1976). The quantitative determination of carotenoids was conducted based on the extinction coefficient at the maximum absorption wavelength in hexane (Davies 1976).

Statistical analysis was performed by comparing male and female whitefish with the two-tailed Mann-Whitney U-test.

RESULTS

The total concentration of carotenoids in the skin, muscle, and livers of whitefish from Lake Łebsko is presented in Figures 1-3. The levels of carotenoids varied among the analyzed tissues. The lowest content was determined in the skin (0.39-0.41 μg g^{-1} wet weight (ww)). Sex did not have a statistically significant influence on the content of carotenoids in the skin (Fig. 1), while the fish muscles contained twice as much of these compounds (0.78 – 0.90 μg g^{-1} ww). The content of carotenoids was significantly higher ($P < 0.05$) in male muscles than in those of females (Fig. 2). The highest levels of total carotenoids (1.36 to 1.55 μg g^{-1} ww) were found in liver tissues, with the total concentration of carotenoids in male livers significantly higher ($P < 0.05$) than that in female livers (Fig. 3).

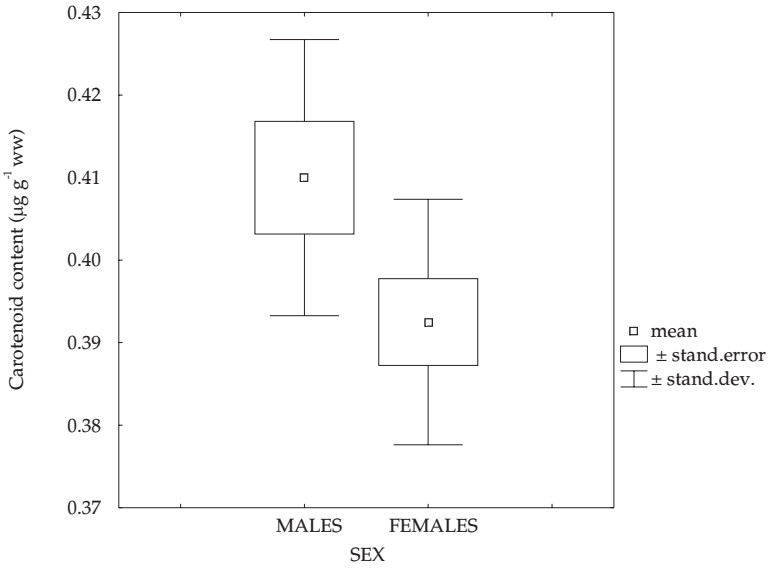

Fig. 1. Total carotenoid content in the skin of whitefish males and females.

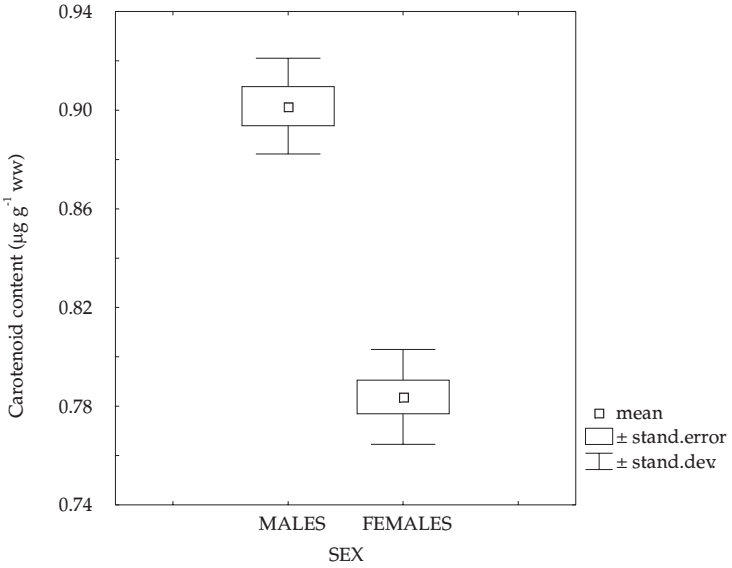

Fig. 2. Total carotenoid content in the muscles of whitefish males and females.

The determinations of particular carotenoids in the skin of the whitefish showed that in both males and females β-carotene was present in the highest percentages, accounting for over 30% of the whole pool of carotenoids (Table 1). Astaxanthin and

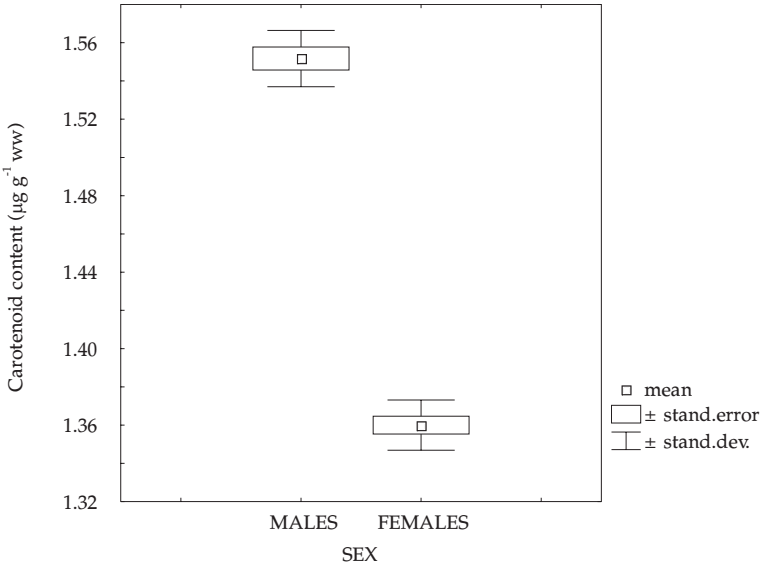

Fig. 3. Total carotenoid content in the livers of whitefish males and females.

canthaxanthin occurred in much lower percentages (2 and 1%, respectively). Varied percentages of carotenoids were also found in the muscles of fish (Table 1), with astaxanthin dominating the pool of these compounds (30% of the total carotenoids). β-carotene, which prevailed among the carotenoids determined in the skin, constituted only 6.5-7.0% of the total carotenoids present in muscle tissues. Astaxanthin also dominated among the carotenoids found in the liver tissues of whitefish (Table 1), in which it ranged from 28 to 30% of the total pool of carotenoids. Significantly lower percentages of zeaxanthin (20%) and canthaxanthin (14.5-15.5%) were determined in liver tissues, in which the level of β-carotene was the lowest.

TABLE 1

Carotenoid content in tissues studied from Lake Łebsko whitefish (percentage of total caretonoid content)

Carotenoids	Skin		Muscles		Liver	
	Female	Male	Female	Male	Female	Male
β-carotene	36.8	37.0	7.0	6.5	7.8	8.0
Lutein	25.0	25.6	20.5	20.9	16.1	16.0
Astaxanthin	2.3	2.0	29.6	31.8	28.0	30.0
Canthaxanthin	1.0	1.1	17.5	19.9	14.4	15.5
Zeaxanthin	18.9	19.0	17.1	17.0	21.3	22.0
Other carotenoids	16.0	15.3	8.3	3.5	12.4	8.5

DISCUSSION

The study revealed that whitefish males had higher concentrations of total carotenoids in comparison with females (Fig. 1). Czeczuga et al. (1991) discovered similar sex-related differences among fish specimens. These differences could have resulted from the fish being ready for spawning. In salmonids, carotenoids are transferred from the muscles and other body parts to the gonads prior to the fish reaching sexual maturity (Kitahara 1983, Ando 1986). The results obtained in the present study suggest that this transfer is more intense in females. Another interesting finding was the much higher concentration of carotenoids in liver versus skin and muscle tissue (Figs. 1-3), which was also observed by Czeczuga (1975) in peled, *Coregonus peled* and vendance, *C. albula*, as well as in barbel, *Barbus barbus*, vimba, *Vimba vimba* and tench, *Tinca tinca* (Czeczuga 1992). It can be supposed that the Lake Łebsko whitefish, which received a constant supply of food rich in carotenoids, accumulated these nutrients in the

liver, the main organ for storing carotenoids (Al-Khalifa and Simpson 1988, Guillon et al. 1989).

The concentration of carotenoids in the muscles of whitefish from Lake Łebsko was comparable to that determined in whitefish from Alpine lakes (Czeczuga et al. 1991). Likewise, a similar concentration of carotenoids in muscles and much higher levels of these nutrients in the liver (2.69 μg g^{-1} ww) were determined by Czeczuga (1975) in a study on the whitefish from lakes in northwest Poland. It seems reasonable to claim that the type and quantity of food supplied to fish is linked directly to the level of carotenoids in their bodies. On the other hand, oxygen supply also seems very important (Czeczuga et al. 1991). The data obtained from the present investigation suggest that the oxygen conditions in Lake Łebsko were suitable, which may have had some indirect influence on the concentration of carotenoids in the whitefish sampled here. According to Czeczuga (1992), the type and amount of food supplied to fish has an influence on the type and percentage of carotenoids found in fish tissues. The highest share of β-carotene was determined in the skin of the whitefish from Lake Łebsko (Table 1), whereas the carotenoids present in the muscle and liver tissues were dominated by the share of astaxanthin. Astaxanthin was also found to make up the highest percentage of all carotenoids in a study conducted by Czeczuga et al. (1991). Tissues of other fish species were also determined to contain prevailing percentages of this carotenoid (Foss et al. 1987, Woźniak et al. 1999). Other carotenoids determined in the skin, muscle, and liver tissues of the whitefish netted from Lake Łebsko are canthaxanthin, zeaxanthin, lutein, and β-carotene. The carotenoids determined in the analyzed samples of fish tissues are present in crustaceans and mollusks, which were fed to the common whitefish in this study.

The results allow the authors to make the claim that astaxanthin is a dominant carotenoid in the whitefish living in Lake Łebsko. The content of carotenoids in the analyzed tissue samples implies that the fish lived in water that was well oxygenated and rich in natural food. It can be assumed that the level of carotenoids was appropriate and did not interfere with the metabolic processes occurring in the fish.

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STRESZCZENIE

ZAWARTOŚĆ KAROTENOIDÓW W ORGANIZMIE SIEI (*COREGONUS LAVARETUS LAVARETUS* (L.)) Z JEZIORA ŁEBSKO (PÓŁNOCNA POLSKA)

Celem badań było określenie wpływu płci na zawartość karotenoidów w skórze, mięśniach i wątrobie siei zasiedlającej jezioro Łebsko. Jest to populacja objęta aktualnie programem restytucji.

Zawartość karotenoidów w badanych tkankach była zróżnicowana w zależności od płci. Nie stwierdzono statystycznie istotnego wpływu płci na zawartość karotenoidów w skórze siei (0,39 – 0,41 μg g^{-1} mokrej masy m.m.) (rys. 1). W mięśniach samców poziom karotenoidów wynosił 0,90 μg g^{-1}m.m. i był istotnie wyższy ($P < 0,05$) w porównaniu z zawartością u samic (0,78 μg g^{-1} m.m.) (rys. 2). Najwięcej badanych składników gromadziło się w wątrobie – 1,36 μg g^{-1} u samic i 1,55 μg g^{-1} m.m. u samców. W tym przypadku również stwierdzono statystycznie istotne różnice ($P < 0,05$) (rys. 3). W mięśniach i wątrobie siei dominowała astaksantyna, natomiast w skórze w największym procencie występował β-karoten (tab. 1).