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Chemical nature of xanthophylls in flesh and skin of cultured Arctic char (*Salvelinus alpinus* L.)

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Arctic char (*Salvelinus alpinus* L.) were fed diets containing astaxanthin, canthaxanthin, a mixture of the two, and shrimp-processing discards. The content and chemical nature of carotenoids in the flesh and skin of Arctic char were monitored over a fifteen-week feeding period. Results indicated that carotenoids were absorbed without any chemical change by both the flesh and skin of the char. Carotenoids were deposited in a larger concentration per unit weight in the belly skin, especially for char fed an astaxanthin-containing diet.

INTRODUCTION

Carotenoid pigments are an important source of natural colourants for use in food and also as feed components for cultured salmonids. Fish such as salmon, rainbow trout, and Arctic char are unable to synthesize carotenoids *de novo* and must obtain them from dietary sources. Carotenoids in seafoods are oxygenated derivatives of β-carotene, referred to as xanthophylls (Peterson *et al.*, 1966; Lambertsen & Braekkan, 1971; Spinelli *et al.*, 1974; Choubert & Luquet, 1975; Torrissen *et al.*, 1989; No & Storebakken, 1992; Shahidi *et al.*, 1993; Synowiecki *et al.*, 1993). Approximately 98% of xanthophylls present in shrimp-processing discards were composed of astaxanthin and its mono- and di-esters (Shahidi & Synowiecki, 1991).

The effects of dietary carotenoids on pigmentation of flesh and skin of Arctic char (*Salvelinus alpinus*) as well as the nutritional value of meat have recently been communicated (Shahidi *et al.*, 1993; Synowiecki *et al.*, 1993). It was concluded that a minimum of nine weeks' feeding on xanthophyll-containing diets was necessary to attain adequate pigmentation of Arctic-char flesh. However, the maximum uptake of carotenoids occurred after fifteen weeks of feeding on diets enriched with xanthophylls. The nutritional composition of cultured char was also superior to that of wild char.

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The objectives of the present study were to investigate the chemical nature of pigments present in the flesh and skin of cultured Arctic char during feeding on diets containing astaxanthin, canthaxanthin, a mixture of both, and shrimp discards.

MATERIALS AND METHODS

Fish and feeding regime

Arctic char (*Salvelinus alpinus* L.) used in these experiments were reared from eggs spawned in the Department of Fisheries and Oceans, Aquaculture Research Centre, Winnipeg, Manitoba, Canada. The eyed eggs were transferred to the Institute of Fisheries and Marine Technology, St. John's, Newfoundland, where they were hatched. Arctic-char fry were transferred to the Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland, where they remained until the end of the experiments. The fish were fed on Corey dry-salmon-grower diet (Corey Feed Mills, Fredericton, New Brunswick, Canada) until the experiments on the effects of pigmented feeds began. For these studies, Arctic char at the start of the experiments were weighed (500–700 g). They were kept and fed carotenoid-containing diets as described elsewhere (Shahidi *et al.*, 1993).

Extraction of carotenoids

Extraction of carotenoids from a 10-g homogenized sample of flesh or skin of Arctic char was achieved by using acetone. The extracted carotenoids obtained after three extractions were transferred to 50 ml of petroleum ether after additions of 100 ml of water to the mixture. The total concentration of carotenoids in the samples was determined as described by Kanemitsu and Aoe (1958).

Determination of carotenoids

The extracted carotenoids from each sample were then separated on thin-layer chromatographic plates, Silica-gel TCL plates (Analtech Inc., Newark, DE, USA) were spotted with the extracts in chloroform and were then developed in a solvent system consisting of benzene–petroleum ether–acetone (10:3:2, v/v/v). Each component was extracted from the scraped silica gel three times with 3 ml of chloroform. The absorbance of carotenoids in solution, after centrifugation and dilution to 10 ml, was read on a Hewlett–Packard diode-array spectrophotometer. The concentration of individual carotenoids in chloroform was then calculated by using linear regressions of standard curves for each standard carotenoid provided by Hoffmann-La Roche (Ethobicock, Ontario, Canada).

RESULTS AND DISCUSSION

The composition of feed formulations used for Arctic char (*Salvelinus alpinus* L.) is given in Table 1. The content of xanthophylls in the formulation containing shrimp-processing discards was approximately 10% of that in other diets. The chemical nature and content of individual xanthophylls in the flesh and skin of cultured char fed different diets over a fifteen-week period are summarized in Tables 2 and 3, respectively. The chemical structures of pigments found in flesh and skin of Arctic char are given in Fig. 1. A close scrutiny of these results indicates the following trends

(i) The flesh of cultured char at the beginning of experimentation contained 1·35 ± 0·06 μg/g lutein, while their skin contained 10·11 ± 0·32μg/g lutein and 0·62 ± 0·05 μg/g astaxanthin and its esters. The content of lutein, during feeding formulated diets containing carotenoids, decreased progressively.

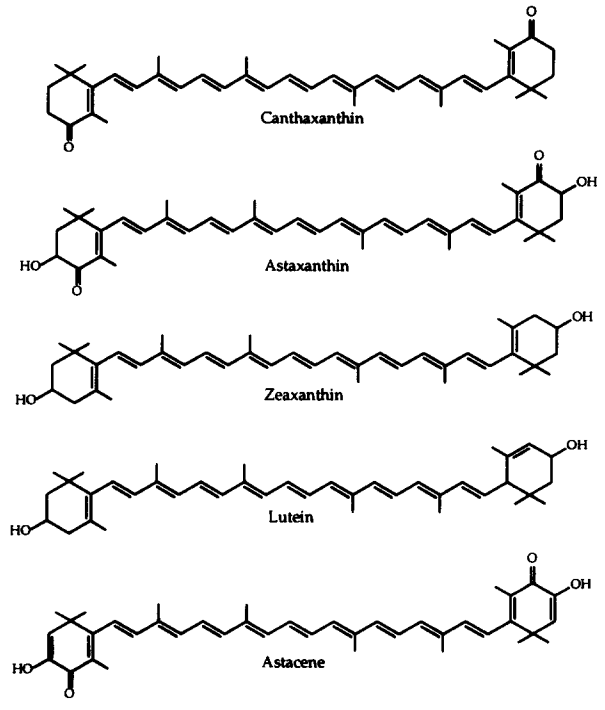

Fig. 1. Chemical structures of xanthophylls in Arctic-char flesh and skin.

(ii) During feeding xanthophyll-containing diets, the total content of carotenoids in the flesh and skin of cultured char was increased.

(iii) The chemical nature of absorbed pigments in the flesh and skin of char resembeled those present in formulated diets.

(iv) The uptake of astaxanthin per unit weight in the belly skin was higher than that in the flesh of char, whereas the opposite was noted for char fed canthaxanthin-containing diets.

(v) The uptake of xanthophylls in the flesh of cultured char exceeded 4 μg/g after a nine-week feeding on all diets, except that containing shrimp offal.

The origin of lutein in the flesh and skin of Arctic char at the beginning of the experiments was traced to corn meal present in the Corey dry-salmonid-grower diet on which the fish were fed. Furthermore, the chemical nature of absorbed pigments by both flesh and skin of char was similar to those present in feeds. Kuo *et al.* (1976) reported that the flesh of rainbow trout fed diets containing red-crab-processing discards showed an increase in the amount of astaxanthin and its esters and a decrease in the content of lutein and zeaxanthin. The uptake of astaxanthin and canthaxanthin has been

Table 1. Proximate composition of feed formulations for Arctic char[a]

Source of feed xanthophylls	Xanthophylls (μg/g)	Moisture (%)	Crude protein (%, dwb)	Lipid (%, dwb)	Ash (%, dwb)	Carbohydrates/fibre (%, dwb)
Astaxanthin	75·0	27·37 ± 0·09	56·3 ± 0·67	15·76 ± 0·03	11·88 ± 0·08	16·01
Canthaxanthin	75·0	35·10 ± 0·09	58·1 ± 0·34	19·67 ± 0·09	12·52 ± 0·09	9·74
Commercial meal	78·3	7·54 ± 0·02	48·2 ± 0·10	26·01 ± 0·08	7·50 ± 0·05	18·31
Shrimp meal (20% in feed)	7·2	47·4 ± 0·16	47·0 ± 0·26	17·47 ± 0·28	15·82 ± 0·32	18·80

[a] Results are mean values of triplicate determinations ± standard deviation. dwb is dry-weight basis.

Table 2. Major xanthophylls of Arctic char fillets (μg/g sample)[a]

Source of feed xanthophylls	Feeding period (weeks)	Astaxanthin and its esters	Canthaxanthin	Lutein	Others
Initial	0	0	0	1·35 ± 0·06	0
Astaxanthin	9	2·99 ± 0·02	0	1·11 ± 0·07	0
	12	3·17 ± 0·10	0	1·39 ± 0·04	0·11 ± 0·02
	15	3·47 ± 0·05	0	1·22 ± 0·10	0
Canthaxanthin	9	0	3·36 ± 0·09	1·50 ± 0·02	0
	12	0	3·46 ± 0·07	1·05 ± 0·06	0
	15	0	4·74 ± 0·03	1·03 ± 0·02	0
Commercial meal (astaxanthin/canthaxanthin, 2:3, w/w)	9	1·40 ± 0·07	1·43 ± 0·10	1·52 ± 0·02	0·05 ± 0·01
	12	1·51 ± 0·09	2·15 ± 0·07	1·49 ± 0·03	0·09 ± 0·01
	15	2·42 ± 0·01	3·12 ± 0·02	0·44 ± 0·01	0·18 ± 0·02
Shrimp meal (20% in feed)	9	0·12 ± 0·02	0	0·95 ± 0·07	0
	12	0·10 ± 0·01	0	1·01 ± 0·02	0
	15	0·36 ± 0·02	0	0·78 ± 0·05	0

[a] Results are mean values of triplicate determinations from four fish ± standard deviation.

Table 3. Major xanthophylls of Arctic char skin (μg/g sample)[a]

Source of feed xanthophylls	Feeding period (weeks)	Astaxanthin and its esters	Canthaxanthin	Lutein and its esters	Others
Initial	0	0·62 ± 0·05	0	10·1 ± 0·32	0
Astaxanthin	9	5·04 ± 0·27	0	11·0 ± 0·21	0·27 ± 0·05
	12	11·5 ± 0·24	0	12·0 ± 0·15	1·31 ± 0·21
	15	34·7 ± 0·59	0	6·12 ± 0·11	0·48 ± 0·19
Canthaxanthin	9	0·89 ± 0·12	13·2 ± 0·19	8·32 ± 0·22	0
	12	0·73 ± 0·03	17·0 ± 0·09	7·21 ± 0·35	0
	15	1·21 ± 0·12	18·2 ± 0·23	5·05 ± 0·17	0
Commercial feed (astaxanthin/canthaxanthin, 2:3, w/w)	9	5·47 ± 0·13	0·90 ± 0·01	12·7 ± 0·22	0·24 ± 0·05
	12	8·08 ± 0·29	1·45 ± 0·12	11·1 ± 0·10	0·14 ± 0·09
	15	10·3 ± 0·23	2·30 ± 0·18	7·78 ± 0·19	0·86 ± 0·12
Shrimp meal (20% in feed)	9	3·11 ± 0·13	0	14·7 ± 0·17	0·38 ± 0·02
	12	4·24 ± 0·15	0	13·4 ± 0·22	0·30 ± 0·04
	15	8·99 ± 0·54	0	9·23 ± 0·97	0·43 ± 0·07

[a] Results are mean values of triplicate determinations from four fish ± standard deviation.

reported to be from ten to twenty times as effective as those of lutein and zeaxanthin (Torrissen *et al.*, 1989).

All fish fed on xanthophyll-containing diets, except those on shrimp offal, acquired over 4 μg/g carotenoids in their flesh. This amount is considered as the minimum required for adequate colour impression of muscle tissues. This concentration was reached after a nine-week feeding on formulated diets. However, longer feeding on these diets resulted in a better uptake of pigments. It is also clear that the use of processing discards of shrimp, as such, requires a much longer feeding period to obtain adequate pigmentation. This is not unexpected, since the content of pigments in diets containing shrimp-processing discards was approximately 10% of that in other diets.

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