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Effects of stocking density on colour characteristics and deposition of carotenoids in cultured Arctic charr (*Salvelinus alpinus*)

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The colour characteristics and deposition of canthaxanthin in cultured Arctic charr (*Salvelinus alpinus*), stocked at 40, 50 and 75 kg/m^3, were studied. Hunter **L, a, b** values of fillets, as such, or in the homogenized tissues, and skin of fish varied significantly among density groups. For belly skin, the numerical values of Hunter colour parameters decreased with increasing stocking density. However, no correlation existed between the total carotenoid contents of fish skin and their Hunter **L, a, b** values. On the other hand, the Hunter **L** values of fillets and homogenized tissues were inversely correlated with their carotenoid contents ($r = -0.9245$ to -0.9844), whereas their Hunter **a** and **b** values were directly correlated with carotenoid contents ($r = 0.9040$ to 0.9824 for **a** and 0.9527 to 0.9924 for **b**).

The content of carotenoids in fillets of charr generally increased with duration of feeding on a pigmented diet, but a decrease was noted in some cases after 16 weeks of experimentation. However, no correlation existed between stocking density and the content of fillet carotenoids ($r = -0.6034$). Canthaxanthin, echinenone, 4′-hydroxyechinenone and lutein were identified in fish fillet and canthaxanthin was the main carotenoid present.

INTRODUCTION

Colour an important sensory attribute of foods and may have a direct effect on the price of seafoods. For example, salmon is often priced according to the intensity of its hue. Therefore, fish farmers must provide the right coloration for cultivated fish in order to satisfy consumer expectation. Colour also has an important function in cooking and thermal processing.

Carotenoids contribute to the yellow, orange, and red colours which are found in the skin, shell and exoskeleton of aquatic species. Carotenoids are indeed the most widespread pigments in nature as they occur in bacteria, yeasts, molds, all green plants, and many animals and perform various functions. They are biosynthesized by photosynthesizing organisms, some heterotrophic bacteria and fungi, but not by animals (Bjerkeng, 1992). However, animals are able to absorb carotenoids from their diet, and deposit them in the unesterified form (Shahidi *et al.*, 1992) in their flesh (Ingemansson *et al.*, 1993), where they bind to actomyosin (Henmi *et al.*, 1989). Some aquatic species are also able to modify dietary carotenoids (Buchecker, 1982).

Many of the more expensive seafoods, such as shrimp, lobster, crab, crayfish, trout, salmon, redfish, red snapper, and tuna have orange–red integument and/or flesh containing carotenoid pigments (Haard, 1992). For species such as shrimp, salmon, rockfish, and snapper, the grading or pricing is directly related to the intensity of their red hue (Sacton, 1986). According to Ostrander and Martinsen (1976), consumers consider colour as the most important factor distinguishing salmon and trout. Pen-reared salmon, which lacked typical salmon colour, was not considered to be different from trout by sensory panels.

Flesh pigmentation is particularly important in salmonid species that normally accumulate carotenoid pigments from their natural diet (Gentles & Haard, 1991). Boyer & Toever (1993) suggested that one of the primary retail market requirements in selling Arctic charr (*Salvelinus alpinus*) relates to flesh pigmentation; highly pigmented, red-fleshed fish commanded a high price.

Tacon (1981) argued that, so far, no adequate data have shown a specific function of carotenoids in reproduction; however, Torrissen (1984) found that

astaxanthin or canthaxanthin supplementation in the diet had a growth promoting effect in Atlantic salmon fry. Srivastava (1991) also reported a direct association between carotenoids and the hatching success and survival of alevins of Arctic charr and Atlantic salmon. The present paper reports on the colour characteristics and carotenoids content in the flesh and skin of cultured charr, stocked at 40, 50 and 75 kg/m^3 and fed on a canthaxanthin-supplemented diet.

MATERIALS AND METHODS

Fish and rearing conditions

Two-year old Fraser river strain of Arctic charr (*Salvelinus alpinus* L.), grown in Daniel's Harbour hatchery in Newfoundland, was employed in these studies. Charr from the same population, each weighing approximately 150 g, were divided into three density groups of 40, 50, and 75 kg/m^3. Each group density was stocked into two 2.4 m^3 tanks (i.e. in duplicate) and reared for approximately 24 weeks. Tanks were supplied with fresh water at 5.5–6.6°C at a rate of 15 L/min. All groups were fed manually on a commercial feed pigmented with 65 μg/g canthaxanthin (Moore–Clark Co., St. Andrews, NB) (see Table 1 for details). Ration were set at a level of 4.1% of biomass/day (Fagerlund *et al.*, 1981) and were provided three times a day, six days a week. Feed level calculation was performed separately for each tank. Feed level was adjusted by weighing fish from each tank every three weeks over a 24 week period and water volume was adjusted to maintain the original densities. This approach was adopted because regular removal of individuals could disturb the social relationship within the treatment groups, if adjustment were not made (Laidley & Leatherland, 1988; Baardvik & Jobling, 1990). Fish were sampled every 4–6 weeks from the onset of rearing experiments. Five to six fish from each tank were taken, slaughtered, and immediately frozen and stored at approximately −20°C until used for analyses.

Table 1. Composition of the experimental Arctic charr feed[1]

Feed components	Proportion, %
Moisture[a]	6.1
Crude protein[a]	45.3
Crude lipid[a]	22.6
Ash[a]	8.5
Crude carbohydrate[b]	12.5
Calcium, actual[c]	2.2
Phosphorus, actual[c]	1.5
Sodium, actual[c]	0.3
Vitamin premix, actual[c]	0.5
Mineral premix, actual[c]	0.5
Canthaxanthin pigment[d]	66.87 μg/g

[1]Ingredients: Fish meal, canola meal, soybean meal, feather meal, whole wheat, fish oil, cane molasses, sugar beet extract. A vitamin premix containing retinyl acetate (A), retinyl palmitate (A), vitamin D3, DL-alpha-tocopheryl acetate (E), calcium D-pantothenate, riboflavin, nicotinic acid, thiamine mononitrate, pyridoxin hydrochloride (B6), vitamin B12, D-biotin, folic acid, inositol, menadione dimethyl-pyrimidinol bisulphate (K), ascorbyl polyphasphate (C) was used. A mineral premix containing manganese sulphate, zinc sulphate, calcium iodate, and betaine was used.
[a]Determined in our laboratory.
[b]Calculated by difference.
[c]Based on data provided by the feed manufacturer.
[d]Determined in our laboratory.

Colour measurements

Hunter colour parameters (Hunter **L, a, b**) of the belly skin of fish, the fillet and the homogenized tissues were measured colorimetrically using a Colormet calorimeter (Instrumar Engineering Ltd., St. John's, NF) as described by Shahidi *et al.* (1992). The unit was standardized with a B-143 white calibration tile having a Hunter **L** value of 94.5 + 0.2, **a** value of −1.0 ± 0.1, and **b** value of 0.0 ± 0.2.

Total pigment determination

The method employed for determination of total flesh pigments was that of Saito and Regier (1971). All experiments were carried out under a yellow light and, when possible, under a blanket of nitrogen. Approximately 10 g of the pre-homogenized fish tissues were extracted three times, each with 50 ml of acetone (BDH Inc., Toronto, ON) for 2 min using a Polytron PT 3000 (Brinkniann Instruments, Rexdale, ON) homogenizer at 10 000 rpm. The homogenized sample was then filterred through a Whatman No. 4 filter paper. The filtrate was transferred into a 50 ml tube and centrifuged (IEC Centra MP4 centrifuge, International Equipment Co., Needham, Heights, MA) for 5 min at 4000 ***g***. Carotenoid pigments in acetone were then transferred to 40 ml petroleum ether (Fisher Scientific Co., Fair Lawn, NJ) in a 250 ml separatory funnel. To maximize the transfer of carotenoids, 100 ml of distilled water containing approximately 0.5% (w/v) sodium chloride were added to the mixture. The petroleum ether layer was then separated and transferred into a 50 ml volumetric flask, and made up to volume. The absorption spectrum was recorded at 400–600 nm using a diode array spectrophotometer (Hewlett Packard, Model 8452A, Hewlett-Packard (Canada) Ltd., Mississauga, ON). Total carotenoids in samples were calculated, as canthaxanthin equivalents, according to the equation given below (Simpson *et al.*, 1981), using an extinction coefficient $E^{1\%}_{1\,cm}$ of 2200 for canthaxanthin in petroleum ether as provided by Bauernfeind (1981).

$$C(\mu g/g) = \frac{A_{466\ nm} x V_{extract}}{E^{1\%}_{1\,cm} x W_{sample}}$$

where C = total carotenoid concentration; A = absorbance at 466 nm; V = volume of the extract (ml);

E = extinction coefficient of 1% standard canthaxanthin in petroleum ether in 1 cm cell; and W = weight of tissue extracted (g wet weight). This procedure was also employed for determination of feed carotenoids.

For determination of skin carotenoids, samples were extracted into chloroform–methanol–water (Bligh & Dyer, 1959). All skin, except the head skin, but including all fins, were cut into small pieces using a pair of scissors before the extraction. Approximately 10 g of the cut and homogenized skin were extracted with 25 ml chloroform and 50 ml methanol for about 2 min using a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON) homogenizer at 16 000 rpm. To the mixture, 25 ml of additional chloroform were added and after blending for about 1 min, 15 ml of distilled water were added followed by blending for another 30 s. The homogenate was then filtered through a Whatman No. 1 filter paper on a Buchner funnel with slight suction. The slurry was washed with 35 ml of chloroform and filtered as described above. The filtrate was transferred to a 250 ml separatory funnel, and after complete phase separation, the chloroform layer was removed and the solvent evaporated under vacuum. The carotenoids were then dissolved in 50 ml of petroleum ether. The absorption spectrum of the pigment was recorded at 400–600 nm and the concentration of carotenoids was calculated in a similar manner to that described for flesh carotenoids.

Individual pigments

Determination of individual pigments was carried out for charr flesh. Carotenoids were separated on an aluminium oxide (J. T. Baker Chemical Co., Phillipsburg, NJ) column (15 cm×1.0 cm i.d.). For a better separation of 4′-hydroxyechinenone, it was necessary to use a silica gel G (Selecto Inc., Kennesaw, GA) column (15 cm×1.0 cm i.d.). The pigment extract for total pigment determination was evaporated under vacuum (Büchi 461 Water Bath), and then subjected to the column. A mixture of acetone and hexane was used for elution; the proportions of acetone in hexane were 0, 4, 10, 15, 20, 30, and 50% (v/v). Finally, an acetone–methanol–hexane (1:1:8, v/v/v) solvent system was used to elute the yellow non-carotenoid fraction. Each fraction was collected and the solvent evaporated under nitrogen to dryness. Dried carotenoids were then dissolved in a known volume of petroleum ether, the absorbance of which was read at 400–600 nm. The type of carotenoids in each fraction was identified according to its absorption maximum, and its concentration was determined using the above equation. To confirm the type of carotenoids present, the R_f values of separated pigments on thin layer chromatographic (TLC) plates were compared to those of the standard carotenoids (Hoffman–La-Roche, Etobicoke, ON) and absorption maxima given by Bauernfeind (1981). The carotenoid extract was spotted on a silica gel G TLC plate (Uniplate, Analtech Inc., Newark, DE) and then developed in a benzene–petroleum ether–acetone (10:3:2, v/v/v) solvent system. Each fraction band was then compared with the band of the standard pigment.

RESULTS AND DISCUSSION

Colour parameters

The Hunter **L, a, b** values of belly skin, fillet and homogenized tissues of reared Arctic charr are given in Table 2. The Hunter **L, a, b** values varied significantly ($p < 0.05$) among density groups. For belly skin of fish, colour parameters decreased marginally, but significantly ($p < 0.05$), with increasing stocking density. The fillet and homogenized tissues of fish at a density of 75 kg/m^3 were found to have the highest Hunter **L** value, followed by fish at densities of 40 and 50 kg/m^3, respectively. However, intact fish fillets and homogenized tissues at a density of 50 kg/m^3 exhibited the highest Hunter **a** and **b** values followed by fish at densities of 40 and 75 kg/m^3, respectively (Tables 3–5).

No relationship existed between the Hunter **L, a, b** values of fish belly and carotenoid concentrations

Table 2. The Hunter L, a, b values of belly skin, B; fillets, F; and homogenized tissues, H of Arctic charr reared at different stocking densities at the end of the experiments[1]

Hunter value		Stocking density, kg/m^3		
		40	50	75
L:	B	79.44 ± 0.14^a	$78.66 + 0.94^b$	$77.70 + 0.60^c$
	F	$48.52 + 1.66^a$	$45.65 + 2.64^b$	$48.73 + 2.21^a$
	H	$69.34 + 0.47^a$	$65.76 + 3.34^b$	$71.55 + 0.66^c$
a:	B	$-1.26 + 0.07^a$	$-1.29 + 0.40^{ab}$	$-1.36 + 0.01^b$
	F	14.51 ± 1.23^a	$15.08 + 0.16^a$	$12.40 + 1.13^b$
	H	5.78 ± 0.03^a	$6.55 + 0.68^b$	$4.58 + 0.40^c$
b:	B	$6.02 + 3.22^a$	$5.97 + 1.19^a$	$4.46 + 0.58^b$
	F	21.78 ± 1.64^a	$23.17 + 0.50^a$	$20.87 + 1.05^a$
	H	18.77 ± 0.43^a	$19.33 + 0.73^a$	$17.02 + 0.63^b$

[1]Results are mean values of 30 determinations ± standard deviation.
Values in each row with the same superscript are not significantly different ($p > 0.05$) from one another.

Table 3. Hunter L, a, b values of belly skin of Arctic charr reared at different stocking densities over a 24-week period[1]

	Feeding period, weeks	Density, kg/m^3		
		40	50	75
L:	5	71.67 ± 2.74^a	71.50 ± 2.79^a	72.94 ± 1.13^a
	8	78.33 ± 0.39^a	79.32 ± 0.59^b	78.22 ± 0.72^a
	11	77.13 ± 0.98^a	77.13 ± 0.25^a	79.50 ± 1.47^b
	16	78.41 ± 1.51^a	80.11 ± 0.77^b	80.35 ± 1.23^b
	24	79.44 ± 0.14^a	78.66 ± 0.94^b	77.70 ± 0.60^c
a:	5	-0.93 ± 1.15^a	-1.07 ± 1.43^a	-1.35 ± 0.37^a
	8	-0.77 ± 0.04^{ab}	-1.04 ± 0.25^a	-0.60 ± 0.80^b
	11	-1.36 ± 0.29^a	-1.56 ± 0.71^{ab}	-1.79 ± 0.29^b
	16	-0.37 ± 1.96^a	-1.74 ± 0.34^b	-1.41 ± 0.61^b
	24	-1.26 ± 0.07^a	-1.29 ± 0.40^a	-1.36 ± 0.01^a
b:	5	11.76 ± 4.22^a	10.66 ± 2.80^a	11.30 ± 0.58^a
	8	5.34 ± 1.02^a	5.37 ± 1.25^a	4.97 ± 1.26^a
	11	7.74 ± 1.07^a	5.02 ± 2.82^b	5.93 ± 0.41^b
	16	8.65 ± 2.22^a	8.22 ± 0.78^a	8.38 ± 1.88^a
	24	6.02 ± 3.22^a	5.97 ± 1.19^a	4.46 ± 0.58^b

[1]Results are mean values of 30 determinations ± standard deviation.
Values in each row with the same superscript are not significantly different ($p > 0.05$) from one another.

Table 4. Hunter L, a, b values of fillet of Arctic charr reared at different stocking densities over a 24-week period[1]

	Feeding period, weeks	Density, kg ± m^3		
		40	50	75
L:	5	53.43 ± 1.96^a	51.59 ± 2.25^b	51.94 ± 1.86^b
	8	51.40 ± 3.05^a	50.01 ± 0.65^a	50.60 ± 3.85^a
	11	49.21 ± 0.99^a	49.25 ± 2.60^a	50.55 ± 1.81^b
	16	45.91 ± 1.56^a	47.03 ± 2.08^a	46.61 ± 1.72^a
	24	$48.52 \pm 1.66.$	45.65 ± 2.64^b	48.73 ± 2.21^a
a:	5	9.09 ± 1.87^a	9.22 ± 1.71^a	8.50 ± 1.75^a
	8	10.95 ± 0.88^a	11.47 ± 0.80^a	11.27 ± 1.19^a
	11	11.97 ± 0.17^a	14.14 ± 2.58^b	13.57 ± 1.54^b
	16	14.61 ± 1.96^a	14.59 ± 1.24^a	13.88 ± 1.34^a
	24	14.51 ± 1.23^a	15.08 ± 0.16^a	12.40 ± 1.13^b
b:	5	19.55 ± 0.08^a	20.52 ± 1.39^b	19.87 ± 0.94^{ab}
	8	20.45 ± 0.88^a	21.11 ± 0.05^a	20.82 ± 0.87^a
	11	20.97 ± 0.25^a	21.28 ± 0.50^a	21.06 ± 0.34^a
	16	22.31 ± 1.45^a	21.39 ± 0.76^a	21.23 ± 1.33^a
	24	21.78 ± 1.64^a	23.17 ± 1.10^a	20.87 ± 1.05^a

[1]Results are mean values of 30 determinations ± standard deviation.
Values in each row with the same superscript are not significantly different ($p > 0.05$) from one another.

($r = -0.2450$, -0.1429, -0.2039 for **L**; $r = 0.2092$, -0.6738, -0.8504 for **a**; $r = 0.$ 8386, 0.4820, and -0.1479 for **b** for fish stocked at 40, 50 and 75 kg/m^3, respectively). In contrast, an inverse relationship existed between Hunter **L** values and the content of carotenoids in the intact and homogenized fillets for all density groups ($r = -0.9844$, -0.9480 and -0.9245 for fish stocked at 40, 50 and 75 kg/m^3, respectively). However, their Hunter **a** and **b** values exhibited a direct relationship with carotenoid contents ($r = 0.9514$, 0.9824, 0.9040 for **a**, and $r = 0.9924$, 0.9527 and 0.9558 for **b** for fish stocked at 40, 50 and 75 kg/m^3, respectively).

In general, the Hunter **L** (lightness) values of belly, intact and homogenized fillets of fish decreased while their Hunter **a** (redness) and **b** (yellowness) values increased with increasing carotenoid concentrations in the samples. The increase in Hunter **a** values indicates a more reddish hue in fish flesh and lower **L** values show a darker colour.

At the end of the experiment, intact and homogenized fillets of fish stocked at 40 and 75 kg/m^3 showed increased Hunter **L** and decreased Hunter **a** and **b** values as the concentration of carotenoids in the tissues decreased. The results of the present study clearly indicate the existence of a linear relationship between Hunter **L** and **a** values and carotenoid contents of charr flesh. The decrease in Hunter **L** and the increase in Hunter **a** values with increasing carotenoid contents are in good agreement with findings of Saito (1969), Skrede and Storebakken (1986*a*,*b*) and Shahidi *et al.* (1993).

Homogenizing samples prior to colour measurement has been reported to have a definite effect on the colour

Table 5. Hunter L, a, b values of homogenized tissues of Arctic charr reared at different stocking densities over a 24-week period[1]

	Feeding period, weeks	Density, kg/m^3		
		40	50	75
L:	5	74.60 ± 1.16^{a}	73.57 ± 2.37^{ab}	72.71 ± 1.06^{b}
	8	71.23 ± 1.48^{a}	71.56 ± 1.66^{ab}	72.56 ± 2.06^{b}
	11	71.04 ± 0.40^{a}	70.07 ± 0.07^{b}	71.55 ± 0.66^{c}
	16	68.66 ± 0.99^{a}	70.06 ± 1.81^{b}	68.32 ± 1.04^{a}
	24	69.34 ± 0.47^{a}	65.76 ± 3.34^{b}	70.86 ± 1.01^{c}
a:	5	1.90 ± 0.41^{ab}	2.51 ± 2.02^{a}	1.45 ± 0.68^{b}
	8	3.86 ± 0.44^{a}	3.69 ± 0.36^{a}	4.15 ± 0.15^{b}
	11	5.02 ± 0.48^{a}	4.89 ± 1.19^{ab}	4.50 ± 0.41^{b}
	16	6.35 ± 0.70^{a}	5.72 ± 0.50^{b}	5.37 ± 0.65^{b}
	24	5.78 ± 0.03^{a}	6.55 ± 0.68^{b}	4.58 ± 0.40^{c}
b:	5	13.24 ± 1.04^{ab}	14.17 ± 3.01^{a}	12.56 ± 0.99^{b}
	8	16.18 ± 1.47^{a}	15.48 ± 0.16^{a}	13.92 ± 3.69^{b}
	11	16.37 ± 0.59^{a}	16.20 ± 1.00^{a}	16.35 ± 1.18^{a}
	16	19.11 ± 0.72^{a}	18.46 ± 0.55^{b}	17.29 ± 0.98^{c}
	24	18.77 ± 0.43^{a}	19.33 ± 0.73^{b}	17.02 ± 0.63^{c}

[1]Results are mean values of 30 determinations ± standard deviation.
Values in each row with the same superscript are not significantly different ($p > 0.05$) from one another.

Table 6. Total carotenoid contents (μg/g) of flesh and skin of Arctic charr fed on a canthaxanthin-pigmented diet for 24 weeks

	Feeding period, weeks	Stocking density, kg/m^3		
		40	50	75
Flesh[1]:				
	8	2.19 ± 0.59^{ax}	2.60 ± 0.51^{ax}	2.50 ± 0.54^{ax}
	11	2.77 ± 0.43^{ax}	3.56 ± 0.85^{by}	3.47 ± 0.41^{by}
	16	5.04 ± 0.70^{bx}	4.48 ± 0.38^{cx}	4.60 ± 0.88^{cx}
	24	4.80 ± 0.99^{bx}	6.08 ± 1.18^{dy}	3.95 ± 0.39^{bz}
Skin[2]:				
	8	5.24 ± 0.30^{ax}	4.79 ± 0.08^{ay}	7.93 ± 0.28^{az}
	16	10.20 ± 2.86^{bx}	8.42 ± 2.13^{bx}	8.82 ± 1.95^{abx}
	24	8.82 ± 0.01^{bx}	9.53 ± 0.84^{bxy}	9.81 ± 0.57^{by}
Initial[3]:	Flesh	0.47 ± 0.10		
	Skin	1.05 ± 0.05		

[1,2,3]Results are mean values of 18, 6 and 3 determinations ± standard deviation, respectively.
Values in each column with the same superscript (a,b,c,d) are not significantly different ($p > 0.05$) from one another.
Values in each row with the same superscript (x,y,z) are not significantly different ($p > 0.05$) from one another.

parameters of salmon flesh (Little & MacKinney, 1969; Schmidt & Cuthbert, 1969; Choubert, 1982; Skrede & Storebakken, 1986*a*). In this study, homogenized fillets possessed higher Hunter **L** and lower Hunter **a** and **b** values than those of their intact counterparts. According to Skrede & Storebakken (1986*a*), this effect was likely to be related to the translucency of intact flesh samples.

Total carotenoid pigments

The content of total carotenoids in fish fillets over the course of experimentation is given in Table 6. The carotenoid levels in fillets increased significantly ($p < 0.05$) from an initial amount of $0.47 + 0.10$ μg/g to 4.80 ± 0.99, $6.08 + 1.18$, and $3.95 + 0.39$ μg/g for fish stocked at densities of 40, 50 and 75 kg/m^3 at the end of the experiments, respectively.

The concentration of carotenoids was increased steadily during the feeding period until a maximum level of 6.08 μg/g was attained at the end of the experiments. Torrissen *et al.* (1989) have reported that approximately 6 μg/g carotenoids in fillets of in trout and 3–4 μg/g in fillets of farmed salmon are sufficient to give a satisfactory visual colour impression to fish. The present experiments on Arctic charr fed on a canthaxanthin-containing diet showed the latter level was reached after 11–16 weeks of feeding.

Accumulation of carotenoids in the flesh of Arctic charr increased linearly up to week 16 ($r = 0.9827$ and $r = 0.9948$) for fish stocked at 40 and 75 kg/m^3, respectively; whereas that of fish at a density of 50 kg/m^3 increased linearly over the entire experimental period ($r = 0.9958$). If one assumes that the weight gain is proportional to feed consumption, the amount of carotenoids ingested should also be proportional

to growth, but for fish stocked at 40 or 75 kg/m^3 this was not observed. Torrissen and Torrissen (1984) and Torrissen *et al.* (1984) have indicated that a decrease in the carotenoid content in fish flesh during the feeding may be due to a saturation effect and found that for Atlantic salmon (*Salmo salar*) this level was 4–5 μg carotenoids per gram of tissue. However, this level might be influenced by genetic factors (Torrissen & Naevdal, 1984), dietary level of carotenoids (Torrissen, 1985), growth rate and size of fish (Torrissen, 1986). Torrissen and Naevdal (1984) reported that rainbow trout accumulated between 5.5 and 6.5 μg carotenoids per gram of tissue after 5–6 months of feeding on a diet supplemented with 50 μg/g of canthaxanthin. This agrees well with the results of the present investigation. The decrease in total carotenoid content in fish flesh after reaching a maximum value may also have originated from initiation of maturation of fish gonads. Fish undergoing sexual maturation are known to mobilize carotenoids from muscles and transfer them to their gonads and skin (Crozier, 1970; Tacon, 1981; Storebakken & No, 1992).

The total carotenoid content of fish skin also increased during the experiment, except for that of fish stocked at 40 kg/m^3 which exhibited a decrease at the end of the study (Table 6). The initial content of carotenoids in the skin of Arctic charr was 1.05 ± 0.05 μg/g. On week 8, the carotenoid contents of fish skin were $5.24 + 0.30$, $4.79 + 0.08$ and 7.93 ± 0.28 μg/g for fish stocked at 40, 50 and 75 kg/m^3, respectively. Fish at a density of 75 kg/m^3 deposited considerably higher amounts of carotenoids in their skin during the early stages of rearing as compared to that of other densities. On week 16, skin of fish from all density groups had a similar content of carotenoids ($p > 0.05$). Furthermore, only a moderate relationship existed between stocking density and the content of skin carotenoids ($r = 0.8826$).

The skin of Arctic charr in this study contained a relatively lower amount of carotenoids (8.82–9.80 μg/g wet tissue) than those of charr belly skin reported previously (Synowiecki *et al.*, 1993), but the amounts were higher than those of rainbow trout reported by Bjerkeng *et al.* (1992). In an earlier study, the weight of charr reached 1539 g (Synowiecki *et al.*, 1993) as compared to that of 514–623 g in this study. Thus, both the dilution effect using all skins instead of belly and larger fish size reaching stage of maturation might be responsible for the observed differences.

Individual carotenoid pigments

The concentration of individual carotenoids in Arctic charr fillets fed on a canthaxanthin-supplemented diet is presented in Table 7. Five fractions of carotenoids of charr flesh were separated. Four fractions exhibited absorption maxima at 456, 466, 454, and 444 nm, respectively. The UV-spectrum of the yellow-coloured 5th fraction, eluted from silica gel column with hexane–acetone–methanol (8:1:1, v/v/v), indicated that it was a non-carotenoid compound and lacked any absorption maximum in the visible range, but had a sharp absorption at 242 nm. The occurrence of a yellow, non-carotenoid, pigment in the flesh of rainbow trout has also been reported by No and Storebakken (1992).

The amounts of echinenone, cantliaxanthin and lutein in fish flesh increased significantly ($p < 0.05$) from $0.28 + 0.02$, $1.51 + 0.13$ and $0.15 + 0.01$ μg/g tissue on week 8 to $0.66 + 0.07$, 3.69 ± 0.39 and 0.23 ± 0.03 μg/g on week 16 for fish stocked at 40 kg/m^3, respectively. Corresponding results for fish stocked at 75 kg/m^3

Table 7. Concentrations (μg/g wet tissue) of carotenoids of Arctic charr flesh fed on a carotenoid-containing diet for 24 weeks[1]

Density/fraction number	R_f value	λ_{max}	Feeding period, weeks			
			8	11	16	24
40 kg/m^3:						
I	0.84	456	0.28 ± 0.02^{aw}	0.36 ± 0.01^{ax}	0.66 ± 0.07^{ay}	0.59 ± 0.09^{ax}
II	0.75	466	1.51 ± 0.13^{ax}	2.00 ± 0.07^{ay}	3.69 ± 0.39^{az}	3.43 ± 0.53^{az}
III	0.41	454	0.25 ± 0.02^{ax}	0.27 ± 0.01^{ax}	0.46 ± 0.05^{ay}	0.58 ± 0.09^{az}
IV	0.28	444	0.15 ± 0.01^{ax}	0.14 ± 0.01^{ax}	0.23 ± 0.03^{ay}	0.18 ± 0.03^{az}
50 kg/m^3:						
I	0.84	456	0.33 ± 0.04^{bw}	0.46 ± 0.06^{bx}	0.58 ± 0.02^{by}	0.69 ± 0.08^{bz}
II	0.75	466	1.80 ± 0.23^{bw}	2.57 ± 0.34^{bx}	3.28 ± 0.13^{by}	3.99 ± 0.45^{bz}
III	0.41	454	0.30 ± 0.04^{by}	0.34 ± 0.06^{by}	0.41 ± 0.02^{bz}	0.68 ± 0.08^{bz}
IV	0.28	444	0.17 ± 0.02^{by}	0.18 ± 0.02^{by}	0.21 ± 0.01^{bz}	0.21 ± 0.02^{bz}
75 kg/m^3:						
I	0.84	456	0.32 ± 0.03^{bw}	0.45 ± 0.02^{bx}	0.60 ± 0.04^{by}	0.49 ± 0.01^{cz}
II	0.75	466	1.73 ± 0.15^{bw}	2.51 ± 0.12^{bx}	3.37 ± 0.25^{by}	2.83 ± 0.04^{cz}
III	0.41	454	0.29 ± 0.02^{bw}	0.34 ± 0.02^{bx}	0.42 ± 0.03^{by}	0.48 ± 0.01^{cz}
IV	0.28	444	0.17 ± 0.01^{bx}	0.18 ± 0.01^{bx}	0.21 ± 0.02^{by}	0.15 ± 0.00^{cz}

[1]Results are mean values of 18 determinations ± standard deviation. Values in each row with the same superscript (w,x,y,z) are not significantly different ($p > 0.05$) from one another.
Values in each column for the same fraction with the same superscript (a,b) are not significantly different ($p > 0.05$) from one another.
Carotenoids in each fraction were I, echinenone; II, canthaxanthin; III, 4′-hydroxyechinenone; and IV, lutein.

increased from 0.32 + 0.03, 1.73 + 0.15 and 0.17 + 0.01 μg/g on week 8 to 0.60 ± 0.04, 3.37 + 0.25 and 0.21 + 0.02 μg/g on week 16, respectively. The amounts of these carotenoids in fish fillets decreased significantly ($p < 0.05$) at the end of the experiment. However, the content of 4′-hydroxyechinenone during the experiment increased significantly ($p < 0.05$) in both density groups. In contrast, fish stocked at 50 kg/m^3 exhibited a significant ($p < 0.05$) increase in all fractions of their flesh carotenoids over the entire rearing periods.

The R_f values of isolated carotenoids were 0.84, 0.75, 0.41, and 0.28, which indicated the presence of one single carotenoid in each fraction, i.e. echinenone, canthaxanthin, 4′-hydroxyechinenone, and lutein, respectively. Tentative identity of each compound was established using standard canthaxanthin and lutein (Hoffman–La-Roche, Etobicoke, ON) and/or by comparison of absorption spectra with those reported by Bauernfeind (1981). The R_f value of the main carotenoid in the flesh of charr (Fraction II) was that of canthaxanthin and its absorption characteristics were also identical to that of canthaxanthin extracted from Carophyll red ($R_f = 0.75$) used in the formulation of fish feed. Arctic charr flesh also contained reductive metabolises of canthaxanthin, i.e. echinenone (Fraction I) and 4′-hydroxyechinenone (Fraction III). Presence of these carotenoids in fish flesh lends futher support to the findings of Schiedt *et al.* (1985) and No & Storebakken (1992) that reductive metabolism of canthaxanthin (β,β-carotene-4,4′-dione) may take place in fish organs. According to Ghidalia (1985), possible intermediates of reductive transformation of canthaxanthin are 4′-hydroxyechinenone (4′hydroxy-β,β-carotene-4-one), echinenone (β,β-carotene-4-one), isocryptoxanthin (β,β-carotene-4-ol), and β-carotene (β,β-carotene), respectively. A portion of echinenone content in the charr flesh might have originated from feed pigments as each grain of feed contained 2.28 μg of echinenone. Conversion of canthaxanthin to its reductive metabolises as a result of extraction is not probable since extraction condition might only encourage oxidation. All carotenoids in charr flesh were in the unesterified form which would allow their binding to actomyosin (Henmi *et al.*, 1989).

The content of canthaxanthin in charr fillets after 24 weeks of feeding experiment reached 3.99 μg/g tissues (approximately 71.6% of the total carotenoids). This is slightly lower than that of charr reported previously (Shahidi *et al.*, 1994) (4.74 μg/g) and that of rainbow trout reported by No & Storebakken (1992), both of which used larger fish (> 500 g) as compared with those in the present study (average of 154 g each). It is well known that larger fish deposit carotenoids more efficiently in their flesh than smaller fish (Torrissen *et al.*, 1989). Existing differences in water temperature, feeding regime, diet formulations and size of fish might be responsible for the observed differences. Presence of similar amounts of lutein (0.15–0.23 μg/g) and smaller amounts of canthaxanthin metabolises (0.48–0.69 μg/g) in fish fillets in this study as compared to those observed by Shahidi *et al.* (1994) (0.14–0.23 and 0.56–1.09 μg/g) might arise from the existing differences in feed composition (i.e. dietary lipids and vitamin E level and lipid quality), stage of sexual maturation, size of fish and storage of fish before carotenoids determination. In vacuum-packed fillets of Arctic charr and rainbow trout, however, as little as 5% of total carotenoids were lost after storage for up to 6 months at −20°C (No & Storebakken, 1992; Synowiecki *et al.*, 1993). β-Carotene, the final reduction product of canthaxanthin, was not detected in the Arctic charr flesh, using the chosen extraction procedure. Lack of hydroxyl and keto groups in β-carotene is responsible for its very low affinity for actomyosin (Storebakken & No, 1992). Therefore, free β-carotene is either transported rapidly from muscle tissues into the skin, where it is deposited or is transformed into vitamin A in the intestinal walls, as reported by Simpson *et al.* (1981).

In summary, the present study indicates that stocking density has an effect on the uptake of dietary carotenoids in fish, the highest amount of pigment was deposited in the flesh of fish stocked at 50 kg/m^3. Meanwhile, there was an increase in the concentration of carotenoids in fish flesh as the duration of feeding of fish on pigmented diets was increased. The dietary carotenoids were partially converted to their reductive metabolises. Thus, in addition to the dietary canthaxanthin, echinenone, 4′-hydroxyechinenone and lutein were also identified in the tissues of reared fish.

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