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Dietary lipid and astaxanthin contents affect the pigmentation of Arctic charr (*Salvelinus alpinus*)

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Abstract

The most important quality parameter of salmonids is the red color of their skin and muscles. In this contribution, the effects of astaxanthin and dietary lipid content on the pigmentation of Arctic charr (*Salvelinus alpinus*) were studied. Charrs were fed on diets containing 40, 60, and 80 ppm synthetic astaxanthin together with 10, 18, and 26% dietary lipids for 24 weeks. Results indicated that the astaxanthin concentration in the belly skin and flesh of fish was strongly correlated with both carotenoid and dietary lipid contents, suggesting a significant interaction between diets and the total carotenoid concentration in the belly skin and flesh. The Hunter color *L** values of the fillet and the belly skin were inversely related to their carotenoid levels, whereas their *a** and *b** values were strongly correlated with the total carotenoid concentration. The apparent digestibility coefficient of carotenoids was directly correlated with the level of dietary lipid but inversely correlated with carotenoid contents. A strong correlation between the content of carotenoids retained in the flesh and their digestibility was observed. Thus, the pigmentation of Arctic charr could improve the overall consumer acceptability as well as nutritional and potential market values of Arctic charr.

Keywords Arctic char, Pigmentation, Dietary lipid, Dietary astaxanthin

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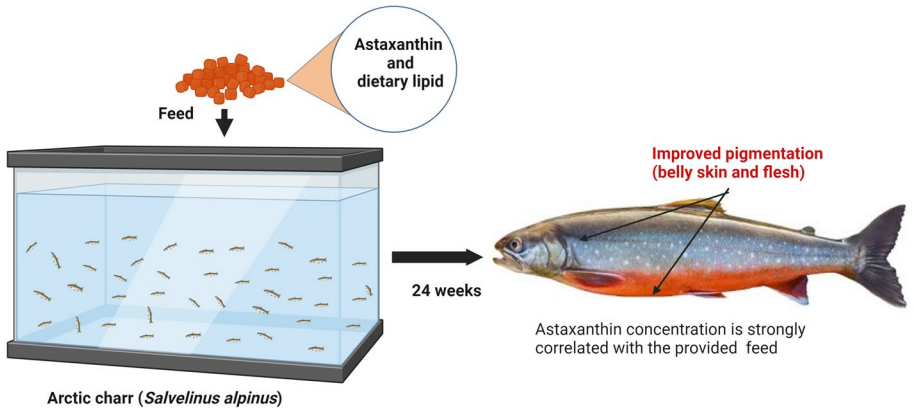
Graphical Abstract



Introduction

The orange, pink, and deep red colors of the skin and flesh of salmonids are a common feature that differentiates this group from other fish species and make a vital impact on their popularity. A satisfactory pigmentation of the flesh of farmed salmonids is fundamental to consumer acceptance. Market values are often at a premium for pigmented fish (Rajasingh et al., 2007; Senadheera et al., 2023). The efficiency of fish muscle actin-binding astaxanthin revealed that the binding efficiency in vitro is similar among different species of fish. Therefore, the difference in the flesh and skin pigmentation is not due to binding capacity in the muscle but rather differences in metabolism and distribution of pigment (Ytrestøyl et al., 2023). In commercial salmonid farming, this pigmentation is achieved by the addition of xanthophylls to the diet. Pigmentation of cultivated salmonid species is influenced by various factors, including the dietary fat and carotenoid levels, source of carotenoids, quality, fish size, state of sexual maturation, and genetic background (Makri et al., 2021; Torrissen et al., 1989). Recent studies suggest that the control of certain gene expressions can have a significant effect on the flesh coloration. The BCO2-1 gene is found to be associated with pigmentation in the flesh, and polymorphism of this gene probably explains the flesh color variation among the salmonids (Lehnert et al., 2019). Another interesting study observed the relationship between water temperature in the summer, Atlantic salmon gut microbiota, and flesh pigmentation and found that gut microbiota, which varies with water temperature, was significantly correlated with the color of the fillet. Compared to pale individuals, darker-fleshed individuals had a higher abundance of carotenoid-synthesizing bacteria in the gut (Nguyen et al., 2020). Of these significant factors, dietary carotenoid levels are a major determinant limiting the concentration of flesh pigmentation in salmonids. However, little information is available to investigate the influence of dietary carotenoid contents on the concentration and retention efficiency of carotenoids in marketable fish (Choubert & Storebakken, 1989; Smith et al., 1992). The observations reported previously about the impact of dietary lipid contents on pigment deposition remain inconsistent (Hien et al., 2022).

There has been an increasing demand for the cultivation of Arctic charr (*Salvelinus alpinus* L.) in the last decade, mainly in Indigenous communities. It serves as a significant local dietary staple, holding considerable cultural significance. Due to the scarcity of adequate information on the precise nutrient requirements of Arctic charr, it is usually fed on commercial feeds, having artificial pigments developed for salmon and rainbow trout (Backstrom et al., 2014; Jobling & Baardvik, 1991). Synthetic pigments such as astaxanthin can significantly increase the redness and yellowness of rainbow trout muscle. Antioxidant capacity of muscle also increased with astaxanthin supplementation, although not in a dose-dependent manner (Kalinowski et al., 2023; Rahman et al., 2016). Astaxanthin-rich red yeast is probably a superior source of red pigment compared to other sources. It can effectively decrease the lipid peroxide generation of tissue, normalize liver function, as well as improve muscle pigmentation (Lim et al., 2017). Compared to salmon and rainbow trout, muscle pigmentation of Arctic charr is challenging to accomplish due to its poor digestibility and pigment retention compared to other salmonids when fed on commercially available

feeds (Christiansen & Wallace, 1988). Nevertheless, the retention of pigment and poor digestibility can be remunerated by increasing the pigment level of the feed. It has been reported that although a linear relationship between flesh and dietary carotenoid concentrations exists in salmon and rainbow trout, the advantage of improving the dietary pigment concentration in feeds reduced at over a specific amount (Smith et al., 1992; Zhao et al., 2022). No matter what the source, the existing natural feed materials high in carotenoid content are expensive and add drastically to the cost of salmonid diets. Thus, it is of economic demand to discover the ideal content of carotenoids in the feed for charr. Furthermore, due to the lipid-soluble characteristic of carotenoids, a carotenoid-rich diet high in lipids is required to accelerate pigment digestion and retention. Therefore, a certain amount of dietary lipid can decrease the amount of dietary pigment needed for reared charr. On the other hand, though the dietary lipid may improve the absorption of pigments, it may have an adverse impact on the retention of carotenoids as lipid free radicals are susceptible to oxidation (Lim et al., 2017). In addition, a high content of dietary lipids could be responsible for an imbalance of the digestible energy ratio to crude protein and more fat deposition in the tissues and visceral cavity, adversely affecting product quality, fish growth, and storage. Any increase in dietary lipids to enhance flesh pigmentation should be optimized to avoid adverse effects on the growth of fish. Thus, it is necessary to discover a suitable dietary lipid level in the pigmented feed for Arctic charr.

To the best of our knowledge, a limited number of studies have been conducted in the late 90 s on the pigmentation of Arctic charr using carotenoids, mainly astaxanthin (Hatlen et al., 1998; Olsen & Mortensen, 1997). Therefore, this study, for the first time, aimed to determine the influences of both carotenoid and dietary lipid levels on the pigmentation of Arctic charr (*Salvelinus alpinus*) harvested from Labrador (Canada) waters. Importance was given to determining the correlation between concentration of carotenoids and dietary lipid levels in the flesh, dietary and flesh carotenoid concentrations, and the interaction between the impact of dietary carotenoid concentrations and dietary lipid levels on the deposition of pigments. The findings of this investigation could address the knowledge gap related to the pigmentation of Arctic charr and potentially other salmonoids.

Materials and methods

Fish (Arctic charr) and culture system

Feeding experiments were performed at the Fisheries and Marine Institute of Memorial University of Newfoundland, St. John's, NL, Canada. Arctic charr, which originated from Frazer River, Labrador, was transferred from Daniel's Harbour Charr Hatchery, Newfoundland, and then kept in a culture system often containing of 80-L plastic tanks attached to a biofiltration-recirculation facility. Fluorescent bulbs were used for lighting on a 10 h light and 14 h dark cycles. Then, 55 charrs were transferred to each 80-L tank and fed on a control diet devoid of carotenoids for 8 weeks before the feeding experiment. During the feeding trial, the mean weight of Arctic charr was 70.16 ± 2.18 g and the culture density was about 40 kg/ m^3. The volume of water in each tank was adjusted from 56.79 to 80.93 L on the 16th week to maintain the density.

Diets

One control diet devoid of carotenoids and 9 experimental diets containing astaxanthin (Carophyll pink, 8%) obtained from Hoffmann-La Roche (Etobicoke, ON, Canada) were produced according to the formulations presented in Tables 1 and 2. The pigment content and proximate composition of the formulated experimental diets are provided in Table 3. The control diet contained small amounts of astaxanthin, which probably originated from the herring oil and herring meal used in the formulation. Nine experimental diets were prepared to contain 40, 60, and 80 ppm of astaxanthin with 10, 18, and 26% of dietary lipids in a 3×3 factorial design.

Table 1 Composition of a control diet (18–0) and nine experimental diets[a]

Ingredients (% of diet)	18–0	10–40	10–60	10–80	18–40	18–60	18–80	26–40	26–60	26–80
Herring meal (crude protein = 67%)	54.9	52.0	52.0	52.0	54.9	54.9	54.9	57.5	57.5	57.5
Whole capelin	25.0	25.0	25.0	25.0	25.0	25.0	25.0	18.0	18.0	18.0
Wheat whole (crude protein = 12%)	5.0	16.2	16.2	16.2	5.0	5.0	5.0	1.0	1.0	1.0
Herring oil	11.1	2.8	2.8	2.8	11.1	11. 1	11.1	19.5	19.5	19.5
Vitamin premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
CRA-VAC binder	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Astaxanthin	0.00	0.005	0. 007	0.009	0.005	0.007	0. 009	0.005	0.007	0.009

[a] The numbers (e.g., 18–0) denote the lipid content (%) followed by the amount of astaxanthin (ppm)

Table 2 Vitamin and mineral content of formulated diets for Arctic charr

Vitamins/ minerals	mg/ kg diet
Vitamin A (as acetate)	9000 I.U.
Vitamin D_3	6000 I.U.
Vitamin E (d1- α-tocopheryl acetate)	375 I.U.
Vitamin K (menadione sodium bisulphite)	45
Thiamin (as HCl salt)	60
Riboflavin	75
Pantothenic acid (as D-calcium salt)	225
Biotin	1.2
Folic acid	22.5
Vitamin B_{12}	0.045
Niacin	300
Pyridoxine (as HCl salt)	45
Ascorbic acid	1125
Inositol	600
Salt (99% NaCl)	3750
Potassium iodine (KI) (76.4% I)	15
Manganous sulphate ($MnSO_4.H_2O$) (32.5% Mn)	525
Ferrous sulphate ($FeSO_4.7H_2O$) (20.1% Fe)	420
Zinc sulphate ($ZnSO_4.7H_2O$) (22.7% Zn)	480
Magnesium sulphate ($MgSO_4.7H_2O$) (9.9% Mg)	525

Pigmentation and sampling of Arctic charr

The control and the nine carotenoid-containing diets were allocated to each group randomly. Arctic charr was fed to satiation twice a day. Moreover, the number of fish, total weight, and feed intake per tank were evaluated monthly. Before fish were distributed into experimental diet groups, 10 charrs were taken as the initial sample. At the beginning of the experiment, all fish in each diet treatment were determined in bulk and then on weeks 4, 8, 12, 16, 20, and 24. At each weighing period, 8 or 9 fish were randomly sampled from each diet group. Arctic charr was starved for 72 h prior to sacrifice and sample.

Color measurements

Color measurements were carried out instantly after sampling. The Hunter *L* (lightness)*, *a* (red/green)*, and *b* (blue/yellow)* color parameters of fillets and belly region skin were evaluated using a Colormet colorimeter (Instrumar Engineering Ltd., St. John's, NL, Canada) as described by Hossain et al. (2018).

Chemical analyses

The proximate composition of the diet and fish flesh were analyzed using the AOAC (2000) method. The content of total carotenoids in the feed, feces, flesh, and skin of charr was measured as described by No and Storebakken (1991). Briefly, 5–10 g of samples were extracted using acetone and repeated three times, then mixed in 50 mL of petroleum ether and water after centrifugation. The petroleum ether phase was washed twice in a separatory funnel, followed by filtration. The absorbance of extracts of carotenoids was measured using a diode array spectrophotometer at 470 nm (Hewlett Packard, Model8452A, Hewlett-Packard Ltd., Mississauga, ON, Canada). The content of total carotenoids was assessed in petroleum ether using an extinction coefficient $E^{1\%}_{1\ cm}$ of 2400 for astaxanthin, based on the method described by Simpson et al. (1981) and Onodenalore et al. (2024).

$$C(\mu g/g) = (A_{470} \times V_{extract})/\left(E^{1\%}_{1cm} \times W_{sample}\right)$$

Where: C = total carotenoid concentration; A_{470} = absorbance at 470 nm; $V_{extract}$ = volume of the extract (mL); $E^{1\%}_{1\ cm}$ = extinction coefficient of 1%

Table 3 Proximate composition (% of feed) and pigment content of the formulated experimental diets for Arctic charr

Diet (% lipid- ppm carotenoid)	Moisture	Crude protein	Total lipids	Ash	Carbo hydrate[a]	Astaxanthin (ppm)
18–0	24.76 ± 0.28	43.03 ± 0.64	18.07 ± 0.07	8.56 ± 0.14	5.58 ± 1.13	6.41 ± 0.23
10–40	24.38 ± 0.28	42.90 ± 0.04	10.33 ± 0.02	8.01 ± 0.58	14.38 ± 1.1	41.23 ± 0.68
10–60	24.59 ± 0.62	41.81 ± 0.66	10.46 ± 0.05	7.97 ± 0.07	15.17 ± 1.4	59.15 ± 0.28
10–80	25.63 ± 0.64	41.05 ± 1.91	9.95 ± 0.08	7.49 ± 0.15	15.88 ± 2.17	78.96 ± 0.40
18–40	23.20 ± 1.21	41.79 ± 0.07	17.73 ± 0.09	7.88 ± 0.09	9.4 ± 0.96	40.26 ± 0.36
18–60	23.79 ± 0.96	42.72 ± 0.02	17.96 ± 0.02	8.23 ± 0.17	7.3 ± 1.17	59.91 ± 0.34
18–80	24.08 ± 0.48	41.04 ± 0.04	18.61 ± 0.08	7.83 ± 0.43	8.44 ± 1.03	79.19 ± 0.25
26–40	19.65 ± 0.52	42.28 ± 0.30	25.82 ± 0.47	7.98 ± 0.38	4.27 ± 1.67	40.89 ± 0.29
26–60	19.51 ± 0.23	42.02 ± 0.02	25.54 ± 0.61	7.57 ± 0.34	5.36 ± 1.2	58.77 ± 0.39
26–80	20.30 ± 0.23	41.06 ± 0.04	26.10 ± 0.37	7.78 ± 0. 54	4.76 ± 1.18	77.95 ± 0.84

Results are mean values of triplicate determinations ± standard deviation

[a] Calculated by difference

standard astaxanthin in petroleum ether in a 1 cm cell; and W_{sample} = weight of tissue extracted (g).

Evaluation of the digestibility of carotenoids

Apparent digestibility coefficients (ADC) of the dietary carotenoids were measured based on Bjerken and Berge (2000) with some modifications. The charr was fed on a diet containing 1% Cr_2O_3 for a week and then starved for a day prior to the stripping of the feces. The chromium oxide level of the diet and feces was analyzed using acid digestion. About 300 mg samples were digested in 5 mL of concentrated nitric acid in a Kjeldahl flask, followed by adding perchloric acid (3 mL). After digestion, the volume was made up to 100 mL with deionized water. The absorbance of the solution was read at 350 nm against deionized water. A standard curve was prepared, where Y = absorbance at 350 nm, and X = chromium oxide content of the sample (mg/ 100 mL).

Apparent digestibility coefficients (%) = $100-[(100 \times \%Cr_2O_3$ in feed $\times$ %Cartenoids in feces)/($\%Cr_2O_3$ in feces $\times$ %Cartenoids in feed)].

Retention of carotenoids

Retention of carotenoids (CR) in the fish flesh was recorded based on the equation described by Zhao et al. (2022).

$$CR = 100 \times [(\mu g \text{ carotenoid increase per g muscle}) / (\mu g \text{ carotenoid supplementation per g fish})]$$

The carotenoid supplementation was determined based on the total consumption of feed and the overall weight of the fish at the end of the experiment.

Specific growth rate

Specific growth rate (SGR %) was assessed as $100 \times (ln$ final weight - *In* initial weight)/ days of feeding, where *In* = natural logarithm (Zhang et al., 2023).

Statistical analyses

The data were presented as mean ± standard deviation of triplicate measurements. The data were subjected to analysis of variance (ANOVA) and regression analysis using SigmaStat™ (v4.0) statistical software.

Results

Growth

The growth data for each diet are presented in Table 4. Arctic charr in all experimental groups grew normally with less than a 1% mortality rate during the whole experiment. The specific growth rate (SGR) of the Arctic charr fed on diets comprising 10% dietary lipids was significantly ($P < 0.05$) lower compared to the fish fed on diets consisting of 18 and 26% lipids, whereas no significant ($P > 0.05$) difference was found between the two groups containing higher contents of lipids. The impact of carotenoid contents on the growth of fish during the trial was not significant ($P > 0.05$).

Body composition

Table 5 presented the proximate composition of the flesh of fish fed on experimental diets for 24 weeks. A significant correlation between the content of dietary lipid and flesh lipid ($r = 0.96$) was observed, while different dietary carotenoid contents did not influence the proximate composition of the flesh ($P > 0.05$). In particular, the moisture content was significantly higher at

Table 4 The growth data of Arctic charr fed diets containing different combinations of dietary lipids and astaxanthin over a 24-week period

Feeding period (week)	Growth data	Diet (%lipid- ppm astaxanthin)									
		18–0	10–40	10–60	10–80	18–40	18–60	18–80	26–40	26–60	26–80
0	Body Weight (g)[1]	66.35	68.75	71.11	70.48	72.16	67.4	71.8	70.66	69.52	73.34
	Length (cm)[2]	16.5 ± 1.5	16.6 ± 1.6	16.6 ± 1.5	16.7 ± 1.0	16.4 ± 1.7	16.3 ± 1.0	16.8 ± 1.1	16.5 ± 1.6	16.4 ± 1.2	16.7 ± 1.0
24	Body Weight (g)[1]	328.2	301.5	321.6	317	367.1	364.7	373.6	386.6	386.8	380.5
	Length (cm)[2]	26.2 ± 1.6	25.7 ± 1.7	26.2 ± 1.6	26.3 ± 1.8	26.8 ± 1.6	27.0 ± 1.8	27.8 ± 1.5	27.3 ± 1.0	28.6 ± 1.0	26.7 ± 1.5
0–24	Weight gain (%)[3]	397.71[a]	338.59[b]	352.31[b]	349.79[b]	408.76[a]	441.08[a]	420. 28[a]	447.17[a]	456.44[a]	418.83[a]
	SGR (Gw%/ day)	0.95[a]	0.88[b]	0.90[b]	0.90[b]	0.97[a]	1.01[a]	0.98[a]	1.01[a]	1.02[a]	0.98[a]

[1] The fish in each tank were weighed in bulk

[2] Results are mean values of 8-10 determination ± standard deviation. Data in each row with different superscript are significantly different ($p < 0.05$) from one another, respectively

[3] Weight gain = (final weight - initial weight)/ initial weight × 100

Table 5 Proximate composition (%) of the flesh of Arctic charr fed on different diets for 24 weeks

Diet (%lipid- ppm carotenoid)	Moisture	Crude protein	Total lipid	Ash
At start:	79.89 ± 0.15^{a}	16.01 ± 1.48^{b}	1.58 ± 0.31^{b}	1.13 ± 0.01^{a}
After 24 weeks:				
18–0	68.50 ± 0.22^{c}	19.89 ± 0.43^{ac}	8.67 ± 0.37^{c}	1.16 ± 0.0^{a}
10–40	70.78 ± 0.07^{d}	20.87 ± 0.13^{a}	5.72 ± 0.23^{d}	1.15 ± 0.03^{a}
10–60	70.06 ± 0.02^{d}	20.57 ± 0.51^{a}	6.39 ± 0.14^{d}	0.68 ± 0.03^{a}
10–80	70.00 ± 0.18^{d}	22.03 ± 1.26^{a}	6.35 ± 0.20^{d}	1.13 ± 0.02^{a}
18–40	68.77 ± 0.33^{c}	20.62 ± 0.48^{ac}	8.11 ± 0.29^{c}	1.08 ± 0.03^{a}
18–60	69.93 ± 0.66^{c}	20.64 ± 0.38^{ac}	7.94 ± 0.16^{c}	0.94 ± 0.05^{a}
18–80	68.71 ± 0.72^{c}	19.53 ± 0.49^{ac}	8.48 ± 0.38^{c}	1.12 ± 0.02^{a}
26–40	67.82 ± 0.30^{b}	18.42 ± 0.43^{c}	10.84 ± 0.41^{a}	1.05 ± 0.01^{a}
26–60	67.11 ± 0.81^{b}	18.97 ± 2.73^{c}	10.48 ± 0.26^{a}	1.04 ± 0.05^{a}
26–80	67.77 ± 0.11^{b}	19.10 ± 0.91^{c}	9.98 ± 0.15^{a}	1.11 ± 0.05^{a}
Dietary lipid	$P < 0.01$	$P < 0.05$	$P < 0.0$ I	$P > 0.05$
Dietary astaxanthin	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Interaction of dietary lipid and astaxanthin	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Results are mean values of triplicate determination ± standard deviation. Values in each column with different superscript are significantly different ($P < 0.05$) from one another

the beginning of the experiment, while the content of crude protein, total lipid, and ash increased throughout the experiment.

Total carotenoid concentration in belly skin and flesh of Arctic charr

The changes in total carotenoid levels in the flesh of Arctic charr during feeding for 24 weeks on experimental diets are presented in Fig. 1. The carotenoid contents in the flesh from all the experimental groups increased significantly ($P < 0.05$) after 24 weeks of feeding, exceeding 4 mg/ kg level (on a wet weight basis), which is generally considered as satisfactory for an acceptable visual color impression of farmed salmon (Torrissen et al., 1989; Ytrestøyl et al., 2023). The key effects (increase) in the carotenoid level of the fish flesh were detected after 8 to 20 weeks of feeding for all treatments. After 20 weeks, the fish flesh carotenoid level stayed comparatively unaffected for the groups fed on diets comprising 10 and 18% lipids but declined in the groups fed 26% lipids in their feed.

After 8 weeks of feeding at each dietary carotenoid content, the total carotenoid level in the flesh enhanced significantly ($P < 0.05$) with increasing the level of dietary lipids. Furthermore, the group containing 26% dietary lipid had the highest rate of increase in the deposition of dietary carotenoids, followed by the group fed 18 and 10% dietary lipids, respectively. The carotenoid contents of flesh in the groups fed 26% lipids exceeded 4 mg/ kg after 12 weeks of feeding at the 60 and 80 ppm of dietary carotenoid levels, while groups containing 10% lipids achieved the similar carotenoid content of over 4 mg/ kg after 20 weeks. The total pigment level in the flesh exceeded 4 mg/ kg on weeks 16, 20, and 24 for groups on 40 ppm dietary carotenoids and 26, 18, and 10% lipids, respectively. However, the level of fish flesh pigments enhanced significantly ($P < 0.05$) as the content of dietary carotenoids increased after feeding on formulated diets containing 18% lipids for 8 weeks. There was a significant ($P < 0.05$) increase in fish flesh carotenoid level when the contents of dietary carotenoid increased from 40 to 60 ppm at the 26% lipid level, but the corresponding increase for 10% lipids was less pronounced at a similar level of carotenoids. After 20 weeks of pigmentation, the changes in the carotenoid content of fish flesh among the three dietary pigment groups were minor but statistically significant ($P < 0.05$) at the 10% dietary lipid level. The fish flesh fed on 80 ppm dietary carotenoids had the highest content of pigment, followed by the group containing 60 and 40 ppm carotenoids, respectively. A significant ($P < 0.05$) interaction was found between the carotenoids and dietary lipids on the content of the total pigment of fish flesh. The trends of the impact of lipids on the content of total carotenoids in the belly skin were like that in the flesh of fish (Fig. 2). A significant ($P < 0.05$) correlation of carotenoids and dietary lipids on the content of pigment of belly skin was also noted.

Correlation between fat content and carotenoid concentration in flesh

There was a strong ($P < 0.001$) correlation ($r = 0.92$) between the total fat (%) and total carotenoid (mg/ kg) contents in charr flesh.

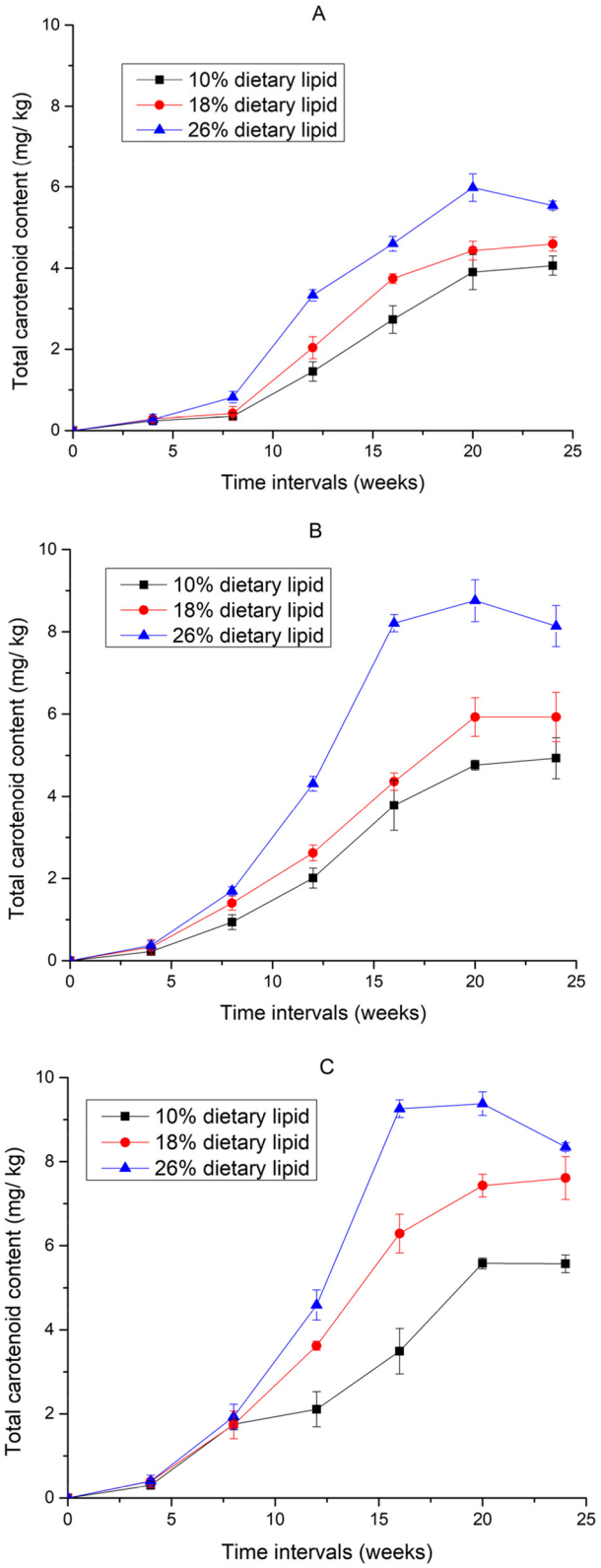


Fig. 1 Total carotenoid content (mg/ kg, on wet-weight basis) in the flesh of Arctic charr fed experimental diets over a 24-week feeding period. **A**, **B**, and **C** stand for 40, 60, and 80 ppm dietary carotenoids, respectively

Correlation between carotenoid concentration in belly skin, flesh, and dietary lipid and carotenoid contents

A direct correlation ($r=0.96$) was found between the carotenoid concentration in charr flesh and both dietary lipid ($P=0.0022$) and carotenoid ($P=0.0008$) levels through multiple linear regression. A similar relationship ($P=0.003$) occurred between carotenoid concentration in the belly skin and carotenoid and dietary lipid contents.

Hunter color values of fillet and belly skin

The instrumentally evaluated color values of the belly skin and fillet of Arctic charr fed on experimental diets over a 24-week feeding period are presented in Table 6. The color intensity of Hunter a^* (redness) and b^* (yellowness) values of fillet and belly skin enhanced with the progression of the period of feeding on pigmented diets with the exception of the control group, while L^* (lightness) values declined. The regression analysis on pigment levels of the flesh and belly skin of Arctic charr, including the initial and control samples, and the Hunter color values revealed that the carotenoid concentration had a significant ($P<0.001$) impact on color changes as indicated by Hunter L^*, a^*, and b^* values. The r values in regression equations for fillets are 0.96, 0.96, and 0.88 for L^*, a^*, and b^*, respectively, while these were 0.96, 0.89, and 0.89 for belly skin, respectively. However, no significant ($P>0.05$) relationship between any of the lipid content and Hunter color values of the flesh was observed through the regression analysis.

Carotenoid retention and digestibility of Arctic charr

The retention and digestibility of carotenoids in fish fed on experimental diets are presented in Table 7. It is evident that with the increased levels of dietary carotenoids, the carotenoid retention in the flesh reduced significantly ($P<0.05$), while it improved ($P<0.05$) with an increase in the level of dietary lipid. The total content of carotenoids retained in charr flesh was strongly correlated with the content of dietary lipid ($P<0.0001$) but was inversely correlated to the concentration of carotenoids ($P=0.0004$), with $r=0.99$. Similarly, with a decrease in the content of lipids or an increase in the content of carotenoids, the digestibility of carotenoids declined. The digestibility of carotenoids correlated directly ($r=0.93$) with the content

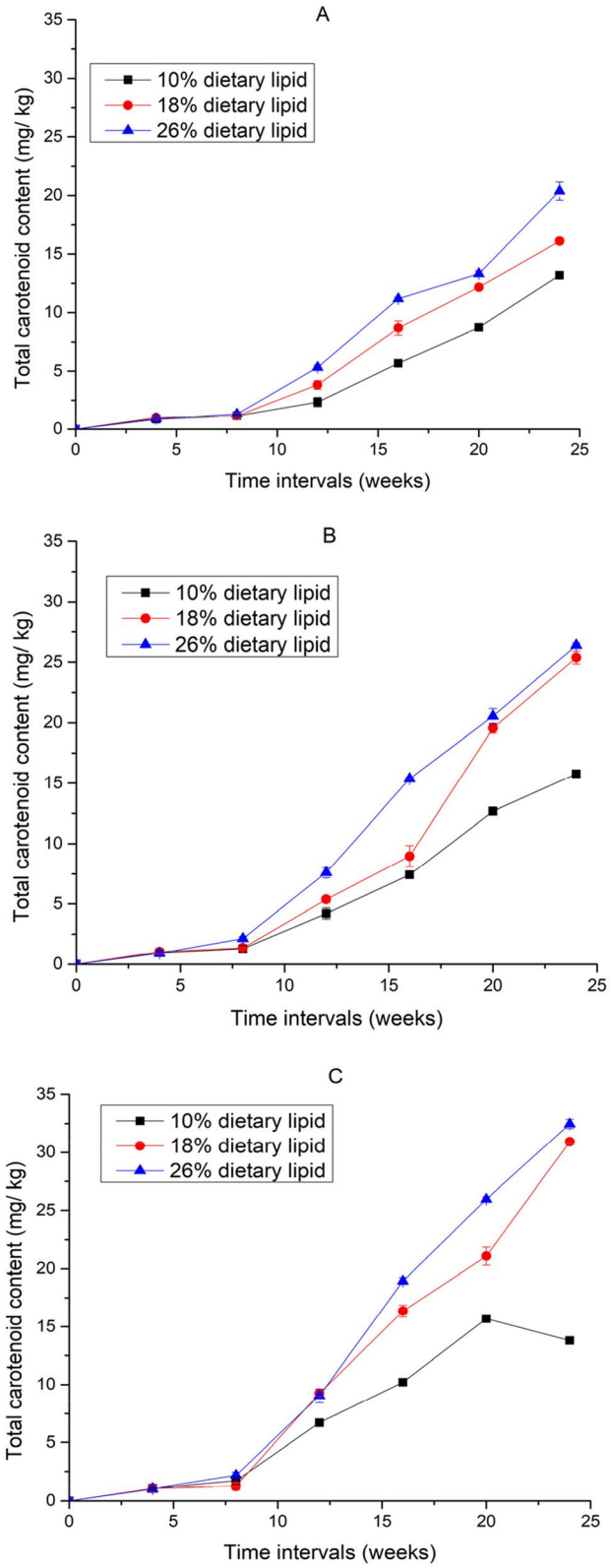


Fig. 2 Total carotenoid content (mg/ kg, on wet-weight basis) in the belly skin of Arctic charr fed experimental diets over a 24-week feeding period. **A**, **B**, and **C** stand for 40, 60, and 80 ppm dietary carotenoids, respectively

of dietary lipid ($P=0.0014$) but was inversely correlated with dietary pigment contents ($P=0.0399$) through the regression analysis of digestibility on the content of dietary lipid and pigment. A relationship ($P=0.0113$) also existed between the digestibility of carotenoids and the content of carotenoids retained in the flesh ($r=0.95$).

Discussions

The mean specific growth rate (SGR) ranged from 0.88 to 1.01% per day over a 24-week of feeding condition, which agrees with previous findings under similar experiments for charr (Christiansen & Wallace, 1988; Zhang et al., 2023). Therefore, the present study did not result in abnormal growth of fish under various levels of dietary lipids and carotenoids. Though there is an indication of the biological activity of carotenoids in fish species, the major purpose of feeding carotenoids to salmonids is to reach an appropriate pigmentation in the product for marketing. In order to be marketable, the flesh of farmed salmonids should have at least 3–4 mg/ kg of total carotenoids (Lim et al., 2017; Torrissen et al., 1989). According to this definition, the final concentration of carotenoids in the flesh of all fish having carotenoids ranged from 3.90 to 9.38 mg/ kg of wet tissue after 20 weeks of feeding, which is satisfactory to maintain the product a natural pink color for marketing. Dietary carotenoid levels, among various factors influencing the carotenoid absorption, retention, and metabolism in salmonids, are the major determining factors that regulate the intensity of flesh pigmentation (Deng et al., 2024; Ytrestøyl et al., 2023). It was found that the diets rich in carotenoids, as compared with their low-level carotenoid counterparts, provided a higher pigment content in the flesh with the increasing of storage time regardless of the content of dietary lipids. Generally, the content of carotenoids in the flesh of fish in several trials exceeded 4 mg/ kg after 12–20 weeks. The longer pigmentation time could be due to the differences in fish size. The fish that were used in that study were about 10-times higher than those in the present study. It has been suggested that the apparent digestibility of dietary carotenoids was greater for large charr than those for small charr (Christiansen & Wallace, 1988). Thus, the content of total carotenoid from all treatments either

Table 6 Hunter *L**, *a**, and *b** values of the fillet and belly skin of Arctic charr after 24 weeks of feeding on experimental diets

Diet (%lipid- ppm carotenoid)	Fillet			Belly Skin		
	*L**	*a**	*b**	*L**	*a**	*b**
At start:	50.38 ± 1.74^{a}	0.28 ± 0.02^{b}	12.33 ± 1.07^{b}	81.06 ± 1.58^{a}	-1.46 ± 0.28^{b}	5.40 ± 1.42^{b}
After 24 weeks:						
18–0	49.69 ± 2.06^{a}	1.21 ± 0.86^{b}	12.39 ± 0.82^{b}	72.05 ± 1.84^{b}	-0.51 ± 0.98^{b}	11.52 ± 2.29^{a}
10–40	44.79 ± 1.51^{b}	15.14 ± 1.01^{ab}	21.61 ± 1.30^{ab}	67.27 ± 5.66^{c}	9.12 ± 2.44^{a}	12.21 ± 1.04^{a}
10–60	43.18 ± 2.59^{b}	16.04 ± 1.33^{ab}	22.16 ± 1.57^{ab}	62.40 ± 2.57^{c}	12.34 ± 2.49^{a}	14.31 ± 1.54^{a}
10–80	39.40 ± 1.10^{c}	17.25 ± 1.20^{a}	23.09 ± 1.03^{a}	63.96 ± 3.99^{c}	12.11 ± 2.60^{a}	15.52 ± 0.81^{a}
18–40	41.62 ± 0.52^{bc}	16.86 ± 1.80^{ab}	23.05 ± 1.93^{a}	66.23 ± 2.58^{c}	9.63 ± 3.92^{a}	13.07 ± 1.05^{a}
18–60	40.27 ± 0.97^{c}	18.37 ± 1.25^{a}	23.99 ± 0.93^{a}	65.15 ± 3.84^{c}	11.90 ± 2.26^{a}	16.82 ± 1.91^{a}
18–80	41.61 ± 1.70^{c}	19.52 ± 2.83^{a}	25.03 ± 1.30^{a}	62.00 ± 6.37^{c}	16.62 ± 3.57^{a}	18.70 ± 1.14^{a}
26–40	43.54 ± 3.14^{b}	17.56 ± 2.09^{a}	3.86 ± 1.86^{c}	65.84 ± 3.77^{c}	12.46 ± 2.52^{a}	14.68 ± 1.53^{a}
26–60	42.19 ± 2.13^{b}	18.73 ± 1.76^{a}	25.90 ± 1.17^{a}	63.54 ± 5.57^{c}	14.72 ± 2.60^{a}	17.43 ± 2.91^{a}
26–80	41.80 ± 2.43^{b}	20.49 ± 2.31^{a}	26.16 ± 1.46^{a}	62.86 ± 5.44^{c}	19.44 ± 4.69^{a}	19.10 ± 2.27^{a}
Dietary lipid	$P > 0.05$	$P < 0.05$	$P < 0.01$	$P > 0.05$	$P < 0.05$	$P < 0.05$
Dietary carotenoid	$P > 0.05$	$P < 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.01$	$P < 0.01$
Interaction of dietary lipid and carotenoid	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Results are mean values of 48 determinations ± standard deviation. Data in each column with different superscript are significantly different ($P < 0.05$)

Table 7 Retention (CR) and apparent digestibility coefficients (ADC) of dietary carotenoid of Arctic charr fed on different diets

Diet (%lipid- ppm astaxanthin)	CR (%)	ADC (%)
10–40	5.56 ± 0.10^{d}	60.33 ± 3.38^{d}
10–60	4.84 ± 0.07^{c}	52.67 ± 2.47^{b}
10–80	4.13 ± 0.29^{b}	47.99 ± 4.10^{b}
18–40	7.67 ± 0.23^{g}	69.55 ± 1.47^{ac}
18–60	6.83 ± 0.19^{f}	67.01 ± 3.16^{ac}
18- 80	6.02 ± 0.07^{c}	65.33 ± 1.33^{c}
26–40	10.66 ± 0.15^{a}	73.62 ± 3.54^{a}
26–60	9.72 ± 0.06^{i}	69.63 ± 2.04^{ac}
26–80	8.16 ± 0.14^{h}	67.10 ± 2.25^{ac}
Dietary lipid	$P < 0.01$	$P < 0.0$ l
Dietary astaxanthin	$P < 0.01$	$P < 0.0$ l
Interaction of dietary lipid and dietary astaxanthin	$P < 0.0$ l	$P > 0.05$

Results are mean values of triplicate determinations standard deviation. Data in each column with different superscript are significantly different ($P < 0.05$)

stayed comparatively constant or decreased after 20 weeks of pigmentation in the flesh of fish, indicating the pigmentation achieved a plateau level. Similar trends were found when Atlantic salmon and rainbow trout (Lim et al., 2017; Ytrestøyl et al., 2023) were used. The decreasing pigmentation level in the trials fed on high dietary lipid contents cannot be justified solely by carotenoid saturation in the body tissues since it can occur in a related fashion for all three dietary carotenoid contents. Furthermore, the declination of pigment level could also be influenced by sexual maturation. It has been suggested that, in male salmonids, the carotenoids are transferred from the flesh to the skin, whereas in females, they are transferred to the gonads (Lim et al., 2017). Serum level of carotenoids decrease in females a month prior to spawning and does not change significantly in males (Gobantes et al., 1998).

Compared to the total content of carotenoids, the retention of pigment (the percentage of consumed carotenoid that is accumulated) in the flesh demonstrated a trend to decline with increasing the concentration of dietary carotenoids. This is in agreement with previous outcomes and strengthens the hypothesis that carotenoid retention in fish is inversely linked to the concentration of dietary carotenoids (Choubert & Storebakken, 1989; Lim et al., 2017). The decline could be due to the constraints in intestinal absorption of carotenoids (Zhao et al., 2022). In addition, the digestibility of carotenoids declined, with improving dietary carotenoids further strengthening the reduced absorption of dietary carotenoids. Different species of salmonids metabolize and deposit astaxanthin and canthaxanthin in a different fashion, which is influenced by the gut anatomy (Page & Davies, 2006) and probably the microbiota within the gut. Except for the limitation in absorption, other factors, including catabolism, limitation in their transport by serum lipoproteins, direction into several tissues, or restricted binding ability to the muscle (carotenoids are bound to actomyosin), could also cause the declining retention of carotenoids (Micah et al., 2022). Supplementation of carotenoids in the diet can change the total lipid profile and hepatic mucopolysaccharide content of the liver in rainbow trout. This

means that supplementation can influence the catabolic potential of the liver in carotenoid metabolism (Cheng & Wu, 2019; Page et al., 2005). Xie et al. (2020) suggested that astaxanthin supplementation alleviated the oxidative stress and inflammation of fish while decreasing the liver weight. This may explain why the increased level of carotenoids correlates with a decreased level of pigmentation (Elbahnaswy & Elshopakey, 2024).

An increase in the lipid level of the diet should assist a better deposition fixation of pigments due to the lipid-soluble nature of carotenoids. As expected, this study indicated that the deposition of carotenoids in the fish flesh improved extensively ($P < 0.05$) with increasing contents of dietary lipids. Similar trends were obtained by other studies on Atlantic Salmon and kuruma shrimp (Wang et al., 2019; Ytrestøyl et al., 2023). This could be due to the higher dietary lipid level in this study, ranging from 10 to 26%. A strong positive correlation ($r = 0.92$) was found between the lipid content of flesh and carotenoid concentration in this study. These findings are contrary to Ytrestøyl et al. (2023) and No and Storebakken (1991) for Atlantic salmon and rainbow trout but agree with the findings of Lim et al. (2017), who observed a positive correlation between salmonid pigmentation and fish body weight. In rainbow trout, a strong positive correlation was found between canthaxanthin and lipid levels in the musculature (Toan et al., 2021). Thus, the existing literature on the pigmentation of salmonids is somewhat ambiguous and fragmentary. So far, there has been no strong dimension of any relationship between carotenoid deposition and lipid distribution in the muscle tissue of salmonids. Consequently, in the present study, the existence of a direct relationship between the dietary lipid content, carotenoid concentration, and lipid level in the fish flesh unambiguously reveals that high dietary lipid levels can enhance both the intestinal absorption of pigments and their deposition in the flesh of charr. The improvement of the apparent digestibility of carotenoids with the dietary lipid level further confirms the enhancement of the metabolism of dietary carotenoids by increasing the dietary lipid content.

The apparent digestibility coefficients (ADC) of astaxanthin in salmonid fish varied between 30–90%, which agrees with the obtained values for Arctic charr (Lim et al., 2017; No & Storebakken, 1991). This could be due to the various types of carotenoids and fish sizes used. Usually, the apparent digestibility of carotenoid is lower for small-size fish than that for large fish, and canthaxanthin is utilized less efficiently than astaxanthin for the pigmentation of salmonid fish (Christiansen & Wallace, 1988; Courtot et al., 2022). The retention of carotenoids in salmonids is widely variable. The retention of carotenoids obtained from this study was valued from 4.13 to 10.67%, which are comparable to those reported by Choubert and Storebakken (1989) and Torrissen et al. (1989). In the present study, the difference between the retention and the apparent digestibility of carotenoids is 40–65%. Due to the low level of carotenoid in the feces, a high apparent digestibility coefficient was observed. This could be due to the decomposition of astaxanthin in the gonads, intestine, and belly skin or metabolization of the absorbed astaxanthin into non-carotenoid components. Besides, the potential incomplete extraction of carotenoids from the feces could have been responsible for this phenomenon. These could automatically lead to lowered rates for carotenoids in the feces and, thus, an overvaluation of apparent digestibility. It is also well known that Cr_2O_3, a marker of digestibility, affects digestibility. Consequently, it is arguable whether traditional digestibility methods can be applied to sensitive molecules such as carotenoids (Christiansen & Wallace, 1988). However, the high correlation ($r = 0.95$) between the retention of carotenoids and digestibility in this study indicates that this technique of digestibility determination still offers a valuable means of evaluating comparative changes in the existence of dietary carotenoids (No & Storebakken, 1991). The results of color analysis in this study revealed a curvilinear relationship among Hunter L^*, a^*, and b^* values and concentration of carotenoids in the belly skin and flesh of charr. This correspondence is similar to that reported for coho salmon, tilapia, and blood parrotfish (Micah et al., 2022; Smith et al., 1992; Tuan Harith et al., 2024). The fact that the intensity of redness (a^* values) improved while lightness (L^* values) reduced concurrently with improving the concentration of carotenoids in the flesh agrees with previous reports (Smith et al., 1992). The curvilinear relationship between the concentration of carotenoids and a value in the fish flesh contrasts with the linear relationship claimed by No and Storebakken (1991). A substantial relationship was also found between the content of carotenoids and yellowness (b^* values) in the fish flesh, and this agrees with the findings of No and Storebakken (1991).

Conclusion

The findings of this study clearly revealed that the increase in both dietary carotenoid and lipid contents in the diet of Arctic charr improved the deposition of carotenoids in their skin and flesh. A significant interaction was found between dietary carotenoids and lipids in the pigmentation of Arctic charr. An increase in dietary lipids within a specific range enhanced flesh pigmentation of Arctic charr. Furthermore, the moisture content of flesh was inversely related to the dietary lipid level, while the protein and mineral content were not significantly influenced. The Hunter L* values were inversely

correlated with their carotenoid contents, whereas their a* and b* values correlated directly with the total carotenoid concentration. Thus, findings of this study help in formulating balanced diet to optimize pigmentation in a cost-saving manner.

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Authors' contributions
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