EFFECT OF DIETARY LIPID AND ASTAXANTHIN LEVEL ON PIGMENTATION OF ARCTIC CHARR (Salvelinus alpinus)

SHUJUN LIN

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EFFECT OF DIETARY LIPID AND ASTAXANTHIN LEVEL ON PIGMENTATION OF ARCTIC CHARR (*Salvelinus alpinus*)

BY

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A thesis submitted to the School of Graduate Studies in partial fulfilment of the requirements for the degree of Master of Science

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ABSTRACT

A 3 × 3 factorial design was used to study the effects of dietary lipid and astaxanthin levels on the pigmentation of Arctic charr (*Salvelinus alpinus*). Different groups of charr with mean initial weight of 70.16 ± 2.18 g were fed with diets containing 10, 18 or 26 % dietary lipid together with 40, 60 or 80 ppm synthetic astaxanthin for 24 weeks. The proximate composition of flesh, total carotenoid concentration in flesh and belly skin, and the Hunter colour values of filets, homogenized flesh and belly skin were determined at 4-week intervals. The apparent digestibility coefficient of carotenoid and specific growth rate (**SGR**) of the experimental fish at different dietary lipid and carotenoid levels were also investigated. Results indicated that:

(1). The moisture content of flesh was inversely related to the level of dietary lipid, while there was a significant direct relationship between the content of flesh and dietary lipid. The protein and mineral content of flesh were not significantly influenced by the level of dietary lipid.

(2). The total carotenoid concentration in flesh and belly skin of charr increased with time. The concentration of carotenoids in the flesh generally reached or exceeded 4 mg/kg wet tissue on week-16, 20 and 24 for the fish receiving 26, 18 and 10 % dietary lipid, respectively. This amount is considered as being sufficient for

providing a satisfactory colour impression. The carotenoid concentration in the flesh and belly skin of fish was directly correlated with both dietary lipid and carotenoid levels. There was a significant interaction between dietary lipid and carotenoid levels on the total carotenoid concentration in flesh and belly skin of charr. A high correlation also existed between the carotenoid concentration and total lipid content in flesh.

(3). The intensity of redness (Hunter **a**) and yellowness (Hunter **b**) of filet, homogenized flesh, and belly skin for all treatments increased with time on pigmented diets while their lightness (Hunter **L**) decreased. The Hunter **L** values of filet, homogenized flesh, and belly skin were inversely correlated with their carotenoid contents, whereas their Hunter **a** and **b** values correlated directly with the total carotenoid concentration. No significant relationship existed between any of the Hunter colour values and the lipid content in the flesh of charr.

(4). The retention of carotenoids in charr flesh varied significantly among treatments, ranged from 4.13 to 10.66 %. The total amount of carotenoids retained in flesh was directly related to the content of dietary lipid, but inversely related to dietary carotenoid levels. There was a significant interaction of dietary lipid and carotenoid levels on the retention of carotenoids in flesh.

(5). The apparent digestibility coefficient of carotenoids, ranging from 47.99

to 73.62 %, correlated directly with the content of dietary lipid, but inversely with dietary carotenoid levels. There was a high correlation between the amount of carotenoids retained in flesh and their digestibility. No interaction of dietary lipid and carotenoid on the carotenoid digestibility was observed.

(6). The dietary lipid and carotenoid levels used in this study did not result in any abnormal growth of fish. The specific growth rate (**SGR**) of the fish fed 18 and 26% dietary lipid were significantly higher than that of the groups receiving 10% dietary lipid. In addition, at the same level of dietary lipid (18 %), the weight gains of fish on dietary carotenoids were faster than that of fish receiving no dietary carotenoids. However, these differences were not statistically significant.

In conclusion, the results of the present study demonstrated that an increase in both lipid and carotenoid levels in charr diet enhances deposition of carotenoids in the fish flesh and skin. There was a significant interaction of dietary lipid and carotenoids on the pigmentation of Arctic charr. Thus, an increase in dietary lipid within a certain range may improve the pigmentation efficacy of charr.

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Chapter 1

INTRODUCTION

1.1 Background and General Review

The pink, orange and deep red colours of the flesh and skin of salmonids are a common characteristic that distinguishes this group from other fish species, and makes a major contribution to their elite image. The degree of flesh pigmentation is one of the most important criteria used by consumers in determining the acceptability of the product (Ostrander and Martinsen, 1976; Sceurman et al., 1979; Gentles and Haard, 1991). Market prices are often at a premium for more highly pigmented fish (Talley, 1991; Shahidi et al., 1993). However, the flesh colour of cultured salmonid fish species may be inferior to that of their wild counterparts due to their diets or husbandry purlieus. This poor pigmentation puts the cultured fish at an economic disadvantage. Thus, it is of importance to provide the "natural" pigmentation to farmed salmonids via dietary means in order to meet the consumer preference for this type of fish.

The pigmentation of wild salmonids is due to the absorption and deposition of oxygenated carotenoids, almost exclusively astaxanthin and its mono- and diesters (Kanemitsu and Aoe, 1958; Matsuno et al., 1980; Schiedt et al., 1981; Kitahara, 1984). Salmonid fish, like other vertebrates, cannot synthesize carotenoids *in vivo* (Hata and Hata, 1973). In nature, salmonids acquire the carotenoid pigments primarily from microcrustaceans and other invertebrates in their diet (Binkowski et al., 1993).

Through various homeostatic mechanisms that appear to be dependent on fish size, sexual maturation and genetic factors, these pigments are both deposited in and eliminated from the muscle tissue (Binkowski et al., 1993). However, farmed fish have no access to carotenoid-containing crustaceans. The failure to provide carotenoid pigments in the diets of cultured salmonids typically results in a pale or very light coloured flesh, which can significantly reduce its market value. Therefore, carotenoids must be added to salmonid's feeds to promote or augment flesh pigmentation in order to inspire its market acceptance.

Torrissen et al. (1989) have discussed several factors which influence carotenoid absorption, retention, and metabolism of cultured salmonids. Among these, dietary levels and sources of carotenoids, dietary lipid levels and quality, and some physiological factors such as fish size, sexual maturation and genetic variations within the stock are considered important. Of these factors, dietary carotenoid levels are the major determinant controlling the intensity of flesh pigmentation in salmonids (Tyczkowski and Hamilton, 1986). Despite its importance, little work has been conducted on the effect of dietary carotenoid levels on pigment concentration and on carotenoid retention efficiency in marketable fish. Previous research had aimed at improving pigmentation by mostly dealing with the pigment source, including those from marine sources (e.g. crustacea and crustacean byproducts) (Peterson et al., 1966;

Lambertsen and Braekkan, 1971; Saito and Regier, 1971; Spinelli et al., 1974; Choubert and Luquet, 1975, 1983; Spinelli and Mahnken, 1978; Kotik et al., 1979; Spinelli, 1979; Torrissen and Braekkan, 1979; Torrissen et al., 1981; Torrissen 1985; Arai et al., 1987), plant and algae (Peterson et al., 1966; Savolainen and Gyllenberg, 1970; Johnson et al., 1980; Sommer et al., 1992; Choubert and Heinrich, 1993), yeast (Johnson et al., 1977, 1980; Gentles and Haard, 1991; Binkowski et al., 1993; Nakano et al., 1995) and synthetic pigments (Schmidt and Baker, 1969; Lambertsen and Braekkan, 1971; Spinelli et al., 1974; Johnson et al., 1980; Schiedt et al., 1981; Luquet et al., 1983; Foss et al., 1984; Storebakken et al., 1987; No and Storebakken, 1992; Smith et al., 1992). However, little work has been conducted on unraveling the relationship between dietary carotenoid levels and pigmentation in salmonids (Choubert and Storebakken, 1989; Smith et al., 1992).

Since carotenoid molecules are lipophilic, a carotenoid-enriched diet which is high in lipids might theoretically be expected to facilitate pigment deposition. It would be interesting to investigate the effect of dietary lipid levels on pigmentation of salmonids. However, the effect of dietary lipid levels on pigment deposition is not clear. Trials using rainbow trout, *Oncorhynchus mykiss*, have indicated a positive correlation between canthaxanthin deposition and lipid levels in muscle tissue, as well as between canthaxanthin deposition and lipid levels in the diet (Abdul-Malak et al.,

1975). Seurman et al. (1979) and Torrissen (1985) have reported that increasing lipid content in the diet significantly promoted deposition of astaxanthin in the flesh, while Spinelli (1979) was able to achieve a 33% increase in flesh astaxanthin by increasing the fat content from 10 to 15% in the diet of rainbow trout. The apparent digestibility of canthaxanthin in rainbow trout was found to increase with increasing level of dietary lipid (Torrissen et al., 1990). However, in another study, researchers failed to demonstrate the existence of any correlation between carotenoid deposition in muscle tissue and dietary lipid levels when the latter was increased from 9.4 to 17.4% (Choubert and Luquet, 1983). McCallum et al. (1987) indicated that there was no significant correlation between the degree of pigmentation and the lipid content of the flesh of chinook salmon (*Oncorhynchus tshawytscha*). No and Storebakken (1991) also reported that the flesh pigmentation of rainbow trout did not significantly relate to the fat content of the flesh. The effect of the type of dietary lipid on the pigmentation of salmonids also remains unclear. Nicolaide and Woodall (1962) initially found impaired pigmentation in chinook salmon fed diets deficient in linoleic (18:2ω6) and linolenic (18:3ω3) acids. Atlantic salmon (*Salmo salar*) had a significantly higher carotenoid content in their flesh when they were fed on a diet high in polyunsaturated fatty acids (**PUFA**) as compared to those lacking **PUFA** (Chritiansen et al., 1993). Anon (1986) also reported that the quantity of canthaxanthin deposited

in trout flesh was influenced by the fat source. It was found that canthaxanthin dissolved in oleic acid was deposited at a higher rate than canthaxanthin dissolved in other fatty acids. Nevertheless, Castell et al. (1972) and Watanabe et al. (1974) indicated that the dietary level of **EFA** (essential fatty acid) was not correlated with pigmentation of rainbow trout. Torrissen (1985) also reported that there was no significant difference in pigmentation between rainbow trout fed on oils "high" in free fatty acids and those fed an oil with a lower content of free fatty acids.

During the last decade there has been an increased interest in the cultivation of Arctic charr (*Salvelinus alpinus* L.). In common with other wild salmonids (Torrissen et al. 1989), the carotenoids found in the tissues of charr are either free astaxanthin or esters thereof, with only free astaxanthin being found in the muscle (Scalia et al. 1989). The muscle of wild charr often looks redder than that of other salmonids, and from the limited data available, it appears that the carotenoid content of charr muscle is towards the higher end of the range for salmonids (Scalia et al., 1989; Torrissen et al., 1989). This red flesh colouration is deemed desirable by the consumer. Recently, the price of deep-red coloured large charr (4-6 lb) greatly exceeded that paid for pale coloured charr (Shahidi et al., 1993). Therefore, attempts have been made to produce farmed charr with the deep-red colour characteristic of the wild fish (Christiansen and Wallace, 1988; Shahidi et al., 1993).

Due to limited information available about the specific nutrient requirements (Jobling, 1991), Arctic charr are usually fed on commercial feeds, containing synthetic pigments, developed for rainbow trout and salmon (Tabachek, 1984). It has been stated that muscle pigmentation in Arctic charr is difficult to achieve when charrs have been fed on commercially available feeds (Refstie, 1981). Christiansen and Wallace (1988) have reported that the muscle tissue of two year old charr contained only 2 mg of pigment per kilogram sample following feeding for 9 weeks on a diet containing 40 mg of canthaxanthin per kilogram, due to their poor digestibility (18% for 1+, and 39% of 2+ fish) and retention of canthaxanthin. This pigment concentration is far below the 3 to 4 mg/kg considered as being an acceptable level in the flesh of marketed salmonids (Torrissen et al., 1989). However, it seems that the poor digestibility and retention of pigment can be compensated by increasing the pigment content of the feed. Shahidi et al. (1993) have found that when Arctic charr (Labrador strain) were fed on formulated feeds containing 75 ppm astaxanthin or canthaxanthin for 9-15 weeks, the level of carotenoid in fillets of fish exceeded 4 mg/kg, which is considered sufficient for visual colour impression of the filets. Therefore, dietary carotenoid levels may play an important role in the pigmentation of Arctic charr.

Although there is a linear relationship between dietary and flesh carotenoid

concentrations in salmonids such as rainbow trout and salmon, the benefit of increasing the dietary pigment concentration in feeds decreases at above a certain amount. Bjerkeng et al. (1990) reported that increasing the amount of dietary carotenoids from 50 to 100 mg/kg for rainbow trout had a minimal effect on enhancing the content of flesh carotenoids as compared to when the increase was from 25 to 50 mg/kg. The overall flesh carotenoid retention efficiencies in relation to dietary carotenoid levels were almost the same for pan-size coho salmon (*Oncorhynchus kisutch*) fed on diets containing 15 mg and 60 mg of astaxanthin/kg, respectively (Smith et al., 1992). Therefore, it is suggested that an economically pigmented diet for pan-size coho salmon would contain approximately 15 mg of astaxanthin/kg. However, little is known about the effect of dietary carotenoid concentrations on the pigmentation of Arctic charr. In addition, because of the lipid-soluble character of carotenoid pigments, a carotenoid-enriched diet high in lipids might be expected, theoretically, to facilitate pigment digestion and retention. As a result, a certain minimum amount of dietary lipid might be expected to reduce the amount of dietary pigment required for reared charr. Since , regardless of source, the commercially available natural feed ingredients high in carotenoid pigments are expensive and add significantly to the cost of salmonid diets, it is of economic significance to find the optimum level of carotenoids in the feed for Arctic charr.

Although dietary lipid may enhance the absorption of pigments by fish (Seurman et al., 1979; Torrissen, 1985; Torrissen et al., 1990), it may have an unfavorable effect on the stability of carotenoids in the feed as lipid free radicals are known to be strong oxidizing agents (Choubert and Luquet, 1983). Moreover, too much dietary lipid may result in an imbalance of the **DE/CP** (i.e. digestible energy/crude protein) ratio and in excessive fat deposition in the visceral cavity and tissues, which would adversely affect fish growth, product quality, and storage (National Research Council, 1993). Any increase in dietary lipid for the purpose of enhancing flesh pigmentation should not affect the growth of fish. Therefore, it is also important to find a proper dietary lipid level in the pigmented feed of Arctic charr.

1.2 Purpose and Experimental Approach

The purpose of this research is to investigate the effects of both dietary lipid and carotenoid levels on the pigmentation of Arctic charr. Emphasis was placed on investigating: (1) the relationship between dietary lipid levels and the flesh concentration of carotenoids, (2) the relationship between dietary and flesh carotenoid concentrations, and (3) the interaction between the effect of dietary lipid levels and the effect of dietary carotenoid concentrations on the deposition of pigments. In addition, the effects of dietary lipid and pigment concentrations on the growth of fish

and the quality of charr flesh were also considered.

To achieve the results to the above considerations, a 3 × 3 factorial feeding experiment was conducted. Fish were fed on diets formulated to contain 10, 18 or 26% lipid with 40, 60 or 80 mg/kg (ppm) carotenoids for 24 weeks. The experimental fish were sampled once a month from the beginning to the end of these experiments. The following parameters were determined for each sample: (1) Colour of flesh; (2) Total carotenoid concentration of flesh and skin; (3) Apparent digestibility of dietary carotenoids; (4) Retention of carotenoids in flesh.

The results of these experiments would provide a comprehensive account of the effect of both dietary lipids and carotenoid concentrations on the pigmentation of Arctic charr. It would also provide a scientific basis for formulating economical diets containing an optimum level of carotenoids for farmed Arctic charr.

Chapter 2

MATERIALS and METHODS

2.1 Feeding Experiments

2.1.1 Experimental Fish and Culture System

Feeding experiments were conducted at the Aquaculture Building of the Institute for Fisheries and Marine Technology of Memorial University of Newfoundland, St. John's, Newfoundland. Experiments lasted 224 days from early October 1995 to late May 1996. Arctic charr, originating from Frazer River, Labrador, with an initial average weight of 36.85 ± 8.72 g were transfered from Daniel's Harbour Charr Hatchery, Newfoundland. Prior to the pigmentation experiment, fish were fed on Biodiet and dry charr grower diet (Moore-Clark Co., St. Andrews, New Brunswick) from start feeding. The fish were maintained in a culture system consisting of ten 80-L plastic tanks connected to a biofiltration-recirculation system. The system was supplied with well water regulated at 12±1°C. Lighting was provided by fluorescent bulbs on a 10 h light : 14 h dark cycle. About 55 charr were transferred to each 80-L tank and fed on a control diet devoid of carotenoids prior to the feeding trial. The fish were acclimatized to this new environment and feed (i.e. control diet) for 8 weeks. When the feeding experiment commenced, the mean weight of fish was 70.16 ± 2.18 g. Culture density was approximately 40 kg/m^3 at the start of the experiment. As density increased, the volume of water in each tank was adjusted from 56.79-L to 80.93-L on the 16th week.

2.1.2 Diets

The formulation and chemical composition of the control diet devoid of carotenoids and nine experimental diets containing astaxanthin are presented in Tables 2.1 to 2.3. The control diet contained small amounts of carotenoids (only astaxanthin), 6.41 ppm on a dry weight basis, probably originating from the herring meal and herring oil used in the formulation. All dietary formulations were based on the above control diet. Nine experimental diets were formulated to contain 10, 18 or 26% dietary lipid with 40, 60 or 80 ppm of astaxanthin in a 3 x 3 factorial design.

In manufacturing the control diet, both vitamin and mineral premixes were ground finer than 0.25 mm before incorporation into the diet. After the dry ingredients had been combined and mixed well, ground whole caplin and fish oil were blended into the mixture. The material was then extruded through a 1/8" die in Hobart food grinder. The extruded material was then manually broken into small pieces, packaged and frozen quickly.

The nine carotenoid-containing diets were prepared by adding a precalculated amount of astaxanthin (Carophyll Pink, 8%), supplied by Hoffmann-La Roche (Etobicoke, Ontario) to provide 40, 60 or 80 ppm pigment to the dry feed ingredients, respectively. The dry components were mixed before being added to the ground whole capelin and herring oil in a feed blender. The pigment (8% water-soluble

Table 2.1 Composition of a Control Diet (18-0) and Nine Experimental Diets[1]

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

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

Table 2.2 Vitamin and mineral content of formulated diets for Arctic charr[1]

Vitamin/Mineral	mg/kg diet
Vitamin A (as Acetate)	9000 I.U.
Vitamin D_3	6000 I.U.
Vitamin E (dl-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

[1]Values for vitamins A, D_3 and E are in I.U.

Table 2.3 Proximate composition (% of feed) and pigment content of the formulated experimental diets for Arctic charr[1]

Diet (% lipid - ppm carotenoid)	Moisture	Crude protein	Total lipids	Ash	Carbo-hydrates[2]	Astaxanthin(ppm, on dry basis)
18 - 0	24.76 ± 0.28	43.03 ± 0.64	18.07 ± 0.07	8.56 ± 0.14	5.58	6.41 ± 0.23
10 - 40	24.38 ± 0.28	42.90 ± 0.04	10.33 ± 0.02	8.01 ± 0.58	14.38	41.23 ± 0.68
10 - 60	24.59 ± 0.62	41.81 ± 0.66	10.46 ± 0.05	7.97 ± 0.07	15.17	59.15 ± 0.28
10 - 80	25.63 ± 0.64	41.05 ± 1.91	9.95 ± 0.08	7.49 ± 0.15	15.88	78.96 ± 0.40
18 - 40	23.20 ± 1.21	41.79 ± 0.07	17.73 ± 0.09	7.88 ± 0.09	9.4	40.26 ± 0.36
18 - 60	23.79 ± 0.96	42.72 ± 0.02	17.96 ± 0.02	8.23 ± 0.17	7.3	59.91 ± 0.34
18 - 80	24.08 ± 0.48	41.04 ± 0.04	18.61 ± 0.08	7.83 ± 0.43	8.44	79.19 ± 0.25
26 - 40	19.65 ± 0.52	42.28 ± 0.30	25.82 ± 0.47	7.98 ± 0.38	4.27	40.89 ± 0.29
26 - 60	19.51 ± 0.23	42.02 ± 0.02	25.54 ± 0.61	7.57 ± 0.34	5.36	58.77 ± 0.39
26 - 80	20.30 ± 0.23	41.06 ± 0.04	26.10 ± 0.37	7.78 ± 0.54	4.76	77.95 ± 0.84

[1]Results are mean values of triplicate determinations ± standard deviation.
[2]Calculated by difference.

astaxanthin in the Carophyll Pink) was dissolved in distilled water at 50°C for 15 min before being added to the diet by spraying it with a spray bottle into the feed mixture. The feed was blended for 5 min and thereafter made to "moist pellets", with a 1/8" diameter, which were vacuum packed in small portions in plastic bags and stored at -40°C. Daily portions were thawed at 5°C for 10 h before being offered to the fish.

All experimental diets were analysed for moisture, crude protein, lipid, ash, carotenoid content and fatty acid composition (Tables 2.3 and 2.4). Based on these analyses, proportions of herring meal, capelin, wheat, fish oil and carotenoids were adjusted to achieve the desired dietary protein, lipid and carotenoid contents.

2.1.3. Pigmentation and Sampling

The control and the nine carotenoid-containing diets were assigned to each fish group at random. Fish were hand fed to satiation (fish no longer actively taking the feed pellets) twice a day. The total weight, number of fish, and feed intake per tank were measured monthly. Ten charrs were taken as the initial sample before fish were distributed into experimental diet groups. All fish in each diet treatment were weighted in bulk at the beginning of the experiment, and then on weeks 4, 8, 12, 16, 20 and 24. Eight or nine fish were randomly sampled from each diet group at each weighting period. Fish were killed by cutting the jugular vein and live-bled in ice water for approximately 10 min. The carcasses were then transferred to the laboratory

Table 2.4 Lipid fatty acid composition of the formulated experimental diets of Arctic charr[1]

Fatty acid composition (%)	Lipid level of diets (%)		
	10	18	26
12:0	0.10 ± 0.04	0.15 ± 0.07	0.12 ± 0.02
14:0	6.90 ± 0.37	6.79 ± 0.54	8.52 ± 0.07
16:0	17.36 ± 0.02	14.49 ± 0.82	14.31 ± 0.17
18:0	3.32 ± 0.52	2.19 ± 0.33	2.04 ± 0.01
20:0	0.24 ± 0.01	0.20 ± 0.01	0.18 ± 0.01
Σ Sat.	27.92 ± 0.21	23.82 ± 0.61	25.16 ± 0.24
16:1ω7	6.64 ± 0.08	8.25 ± 0.58	6.39 ± 0.06
18:1ω9	10.28 ± 0.07	9.46 ± 0.73	11.32 ± 0.04
20:1ω9	9.10 ± 0.81	14.79 ± 0.30	15.32 ± 0.16
22:1ω11	10.48 ± 0.39	20.01 ± 0.29	19.06 ± 0.26
24:1ω9	-	-	0.10 ± 0.14
Σ Mono	36.50 ± 0.67	52.5 ± 0.43	52.18 ± 0.54
18:2ω6	4.50 ± 0.06	3.01 ± 0.04	1.58 ± 0.02
18:3ω6	0.12 ± 0.02	0.13 ± 0.04	0.05 ± 0.07
18:3ω3	0.88 ± 0.01	0.80 ± 0.02	0.82 ± 0.00
20:2ω6	0.21 ± 0.06	0.13 ± 0.18	0.25 ± 0.02
20:3ω3	-	-	0.06 ± 0.08
20:4ω6	1.07 ± 0.12	0.54 ± 0.33	0.64 ± 0.01
20:5ω3	12.03 ± 0.12	8.51 ± 0.42	8.42 ± 0.23
22:5ω3	1.97 ± 0.17	1.16 ± 0.25	1.07 ± 0.04
22:6ω3	14.80 ± 0.20	9.45 ± 0.70	9.80 ± 0.20
Σ Poly	35.58 ± 0.22	23.70 ± 0.83	22.67 ± 0.30
U/S	2.58 ± 0.13	3.22 ± 0.46	2.97 ± 0.04
Σ ω6	5.90 ± 0.33	3.79 ± 0.57	2.51 ± 0.08
Σ ω3	29.68 ± 0.43	19.91 ± 0.39	20.16 ± 0.38
ω6/ω3	0.20 ± 0.02	0.20 ± 0.07	0.12 ± 0.01

[1]Results are mean values of triplicate determination ± standard deviation.

and immediately analysed for the colour of belly region skin and filets. Following colour measurement, fish were separated into flesh, skin (including fin), liver and gut. The respective tissues were then pooled, homogenized, vacuum packed in plastic bags and stored at -80°C until analysed. The skin was frozen without homogenizing. Fish were starved for 72 h before being sacrificed and sampled.

2.2 Colour measurements

Colour measurements were carried out immediately after sampling. The Hunter colour parameters of filets, belly region skin and homogenized tissues were measured with a Colormet colourimeter (Instrumar Engineering Ltd., St. John's, NF) using the Hunter **L, a, b** scale as described by Shahidi et al. (1992). The instrument 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.

2.3 Biochemical Analyses

2.3.1 Proximate Composition

2.3.1.1 Moisture Determination

The moisture content in feed and flesh were determined by oven drying. Three

to five grams of freshly ground sample of feed or 2-3 g of homogenized sample of fish flesh were introduced to a preweighted aluminium pan and then heated in a forced-air convection oven (Fisher Isotemp 300, Fair Lawn, NJ) at 104 °C for 24 h or until a constant weight was reached (AOAC, 1990). Moisture content of the samples was calculated from the weight difference data.

2.3.1.2 Crude Protein Content

Crude protein contents of feed and fish flesh were determined by Kjeldahl method of analysis. One hundred to three hundred milligrams of each sample was heat digested in 20 mL concentrated H_2SO_4 in the presence of a catalyst (Kjeltab M, Profamo, Dorval, PQ) using a Buchi digester for 50-60 min. After digesting, the resultant solution was diluted with 100 ml of distilled H_2O and 150 ml of 25% (w/v) NaOH and distilled in a Buchi 321 distillation unit (Buchi Laboratories, Switzerland). The condensate was collected into a mixture containing 50 ml of 4% (w/v) boric acid solution and 12 drops of a methyl red / methylene blue indicator (Fisher Scientific Co., Fair Lawn, NJ) and subsequently titrated with 0.1 N H_2SO_4 to a red end point. The nitrogen content was calculated and then reported as crude protein content (N% x 6.25) (AOAC , 1990).

2.3.1.3 Total Lipid Content

Total lipids of feed, flesh and liver were extracted using the method of Bligh

and Dyer (1959). Approximate 25 g of sample (for samples with a lower water content of up to 20% such as feed and skin, enough distilled water was added to the sample to obtain an approximate moisture content of 80%, ie. 5-7.5 g sample was used and 20 mL distilled water was added at this point) was homogenized with 25 mL of chloroform (Fisher Scientific Co., Fair Lawn, NJ) and 50 mL of methanol (J.T. Baker Chemical Co., Phillipsburg, NJ) for 2 min using a Polytron PT 3000 homogenizer (Brinkmann Instruments, Rexdale, ON). Another 25 mL of chloroform was then added and the mixture was blended for 30 s. After this, 25 mL of distilled water was added again, and the mixture was homogenized continuously for a further 30 s period.

The homogenate was then filtered through a Whatman No. 1 filter paper (Fisher Scientific Co., Fair Lawn, NJ) using a Buchner funnel with slight suction. The residue and filter paper were re-homogenized with 35.5 mL of chloroform and the slurry was filtered, as described above.

The filtrate was transferred to a 250 mL separatory funnel to separate into two layers (overnight). The chloroform layer (lower phase) containing the purified lipid was then collected and the volume was recorded. A 20 mL aliquot of the chloroform extract was transferred to a preweighed 100 mL round-bottom flask. The solvent was removed under vacuum using a Buchi RE 111 rotavapor. The drying flask containing

lipid residue was placed in a drying oven for 1 h. Then the flask and lipid residue collected was weighted and the total extracted lipids were calculated.

2.3.1.4 Ash Content

Ash content was determined by charring 2-4 g sample in a preweighted porcelain crucible using a Bunsen burner (lower flame) and then heating in a muffle furnace at 550-600°C overnight or until the ash appeared to be creamy white in colour and free of any black particles (AOAC, 1990). The ash content was calculated from the weight difference data.

2.3.2 Fatty Acid Composition of Lipids

The lipid fatty acid composition of charr flesh and feed was analysed by gas chromatography (GC). The method used for transmethylation was similar to that employed by Keough and Kariel (1987). Twenty to thirty milligrams of lipid were placed into a transmethylation vial and transmethylated overnight in an oven (Thelco, Model 2, Precision Scientific Co., Chicago, IL) at 61.8 °C in 6 ml of 6% (v/v) H_2SO_4 in 99.9 mol% methanol containing 15 mg BHA (butylated hydroxyanisole) as an antioxidant. After incubation, 1.0 ml of distilled water was added to the vial and the mixture was extracted three times with 1.5 ml of pesticide-grade hexane. A few crystals of BHA were added again to each vial at the first extraction. The hexane layer was removed into a clean tube and then washed twice with 1.5 ml of distilled H_2O by

vortexing. On the first wash, the H_2O layer was discarded. On the second wash, the hexane layer was transferred into a clean tube. The hexane was then evaporated under N_2 in a fume hood. The dried matter was dissolved in CS_2 prior to GC analysis.

The analysis of fatty acid methyl esters was performed using a Hewlett Packard 5890 gas chromatograph (Hewlett-Packard (Canada) Ltd., Mississauga, ON) on a glass capillary column (30 m x 0.250 mm I.D.) wall-coated with fused silica (film thickness 0.25 μm; Hewlett Packard, Mississauga, ON). Temperature programming was from 220 °C for 10.25 min and then ramped at 30.0 °C/min to 240 °C and held at 240 °C for 9 min. Helium was used as the carrier gas. The temperatures of the injector and the flame-ionization detector were both maintained at 250 °C. Peaks were identified by means of reference standards and comparison with their relative retention times.

2.3.3 Total Carotenoid content

The total content of carotenoids in the skin and flesh of charr was determined as described by Gentle and Haard (1991). A 5 g sample of skin tissue or a 10 g sample of flesh tissue was extracted three times with acetone (BDH Inc., Toronto, ON) for 5 min. The homogenized sample was then filtered through Whatman No. 1 filter paper on a Buchner funnel. The acetone extracts were pooled and mixed with 50 mL distilled water and 50 mL petroleum ether (boiling range, 37.4-52.9; Fisher Scientific

Co., Fair Lawn, NJ) in a separatory funnel. The petroleum ether phase was washed twice with 30 mL distilled water, and the remaining acetone-water phase was re-extracted with petroleum ether until it became colourless. Pooled extracts in petroleum ether were filtered through anhydrous sodium sulphate and dried at 30 °C using a rotovapor. The residue was then dissolved in an appropriate volume of petroleum ether to give an absorbance value in the range of 0.1-0.8. The absorbance was recorded at 470 nm using a diode array spectrophotometer (Hewlett Packard, Model 8452A, Hewlett-Packard (Canada) Ltd., Mississauga, ON). The total content of carotenoids was estimated using an extinction coefficient $\mathbf{E^{1\%}_{1cm}}$ of 2400 for astaxanthin in petroleum ether (Kanemitsu and Aoe, 1958), according to the following equation, as described by Simpson et al. (1981).

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

where $\mathbf{C}$ = total carotenoid concentration; $\mathbf{A_{470}}$ = absorbance at 470 nm; $\mathbf{V_{extract}}$ = volume of the extract (mL); $\mathbf{E^{1\%}_{1cm}}$ = extinction coefficient of 1% standard astaxanthin in petroleum ether in a 1 cm cell; and $\mathbf{W_{sample}}$ = weight of tissue extracted (g wet weight).

For analysis of the total carotenoids in the feed and faeces, 0.5-1 g of freeze-

dried faeces or 5 g of diets were ground in a mortar and suspended in 5 or 15 ml of distilled water, respectively, to make a thin slurry,. The suspension was allowed to stand in a water bath (50 °C) for 30 min with occasional shaking, and then was extracted with 3 x 50 mL acetone as described above (No and Storebakken, 1991).

2.4 Determination of the Digestibility of Carotenoids

Apparent digestibility coefficients (**ADC**) of the dietary carotenoids were determined according to Austreng (1978) using Cr_2O_3 as an inert indicator. On weeks 11, 15, 19 and 23, the charr were fed on a diet containing 1% Cr_2O_3 for 1 week and then starved for 1 day prior to stripping of the faeces. Between 8 and 10 fish from each treatment were lightly anaesthetised with benzocaine and faecal samples were collected from the hind gut region by gently squeezing the ventral abdominal regions. Samples were then pooled, freeze- dried and stored at - 80 °C in dark prior to analysis of chromium oxide and carotenoids.

The chromium oxide content of the diet and faeces was analysed using an acid digestion method (Furukawa and Tsukahara, 1966). Approximately 200-300 mg sample was digested in 5 mL of concentrated nitric acid in a Kjedahl flask until a white precipitate was formed. Perchloric acid (3 mL) was then added to the digestion mixture and the content was reheated until the green colour changed to yellow, orange

or red. 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 using a Hewlett Packard 8452 diode array spectrophotometer (Hewlett-Packard (Canada) Ltd., Mississauga, ON). The standard curve obtained by the wet acid digestion technique may be expressed by the equation: $Y = 0.2089\,X + 0.0032$, where **Y** is the absorbance at 350 nm, and **X** is the chromium oxide content of the sample (mg/100 mL).

Apparent digestibility coefficients (%) were calculated, according to Austemg (1978), as follows:

$$ADC = 100 - (100 \times \frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in faeces}} \times \frac{\%\text{Crotenoids in faeces}}{\%\text{Carotenoids in feed}})$$

2.5 Retention of Carotenoids

Retention of carotenoids (**CR**) in the flesh of fish was calculated according to the following equation (Torrissen and Braellan, 1979):

$$CR = 100 \times \frac{\mu g \text{ Carotenoids increase per g muscle}}{\mu g \text{ Carotenoids supplementation per g fish}}$$

The carotenoid supplementation was calculated from total feed intake and total fish

weight at the termination of the experiment.

2.6 Specific Growth Rate

Specific growth rate (**SGR**) (%) was calculated (Jobling and Wandsvik, 1983) as follows:

$$SGR = 100 \times \frac{ln(final\ weight) - ln(initial\ weight)}{days\ of\ feeding}$$

where **ln** = natural logarithm.

2.7 Statistical Analyses

The data were subjected to analysis of variance (both One-way **ANOVA** and Two-way **ANOVA**) and regression analysis using SigmaStat™ Statistical Software, version 1.03 (Jandel Corporation, 1992-1995).

Chapter 3

RESULTS

3.1 Growth of Arctic charr

Fish in all experimental groups grew normally, with a less than 1% mortality during the trial. The growth data of Arctic charr for each diet are summarized in Table 3.1. After 24 weeks of feeding, the charr, with initial mean weights of 66.35 to 73.34 g, attained a final mean weight of 301.53-386.84 g. There were significant ($P<0.05$) differences between the weight gains of charr on different diets, with lower weight gain observed for groups receiving a lower amount of dietary lipids (10%). However, there were no significant ($P>0.05$) differences in the weight gain between groups receiving 18 and 26 % dietary lipids.

The specific growth rate (**SGR**) of all fish from all treatments decreased generally as fish size increased (Fig. 3.1). The **SGR** of fish fed on diets containing 10% lipid was significantly ($P<0.05$) lower than that of the fish fed on diets containing 18 and 26% lipid, while no significant ($P>0.05$) difference in **SGR** was observed between the two groups on higher amounts of dietary lipids (Table 3.1).

The effect of dietary carotenoid levels on the growth of Arctic charr during the experiment was not significant ($P>0.05$).

Table 3.1 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]	328.24	301.53	321.64	317.01	367.12	364.69	373.56	386.63	386.84	380.51
	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]
0 - 24	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.

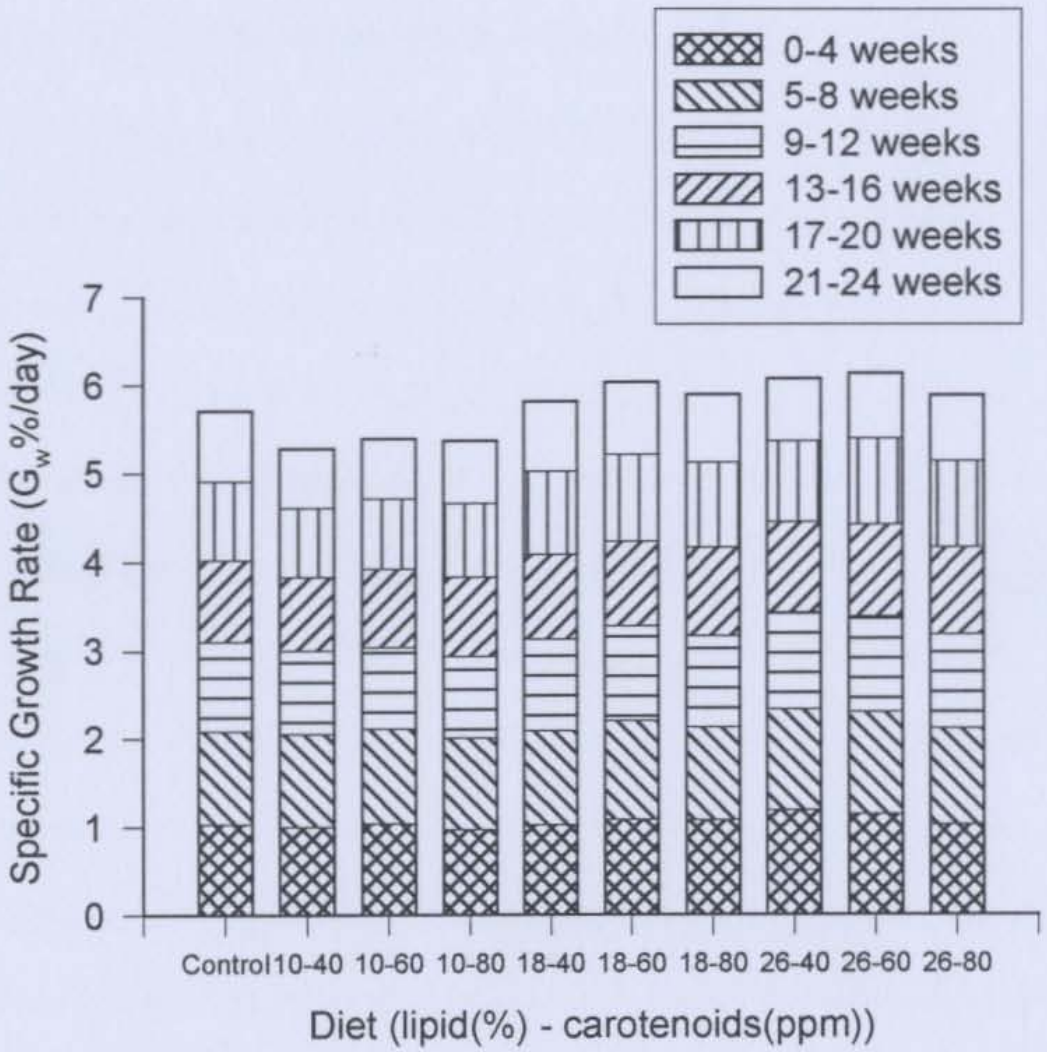

Fig. 3.1 Specific growth rate (G_w%/day) of Arctic charr fed on different experimental diets over a 24-week period

3.2 Body Composition

3.2.1 Proximate composition of flesh

The proximate composition of flesh of Arctic charr fed on experimental diets for 24 weeks is presented in Table 3.2 and Fig. 3.2. As can be seen, the moisture content of fish from all treatments decreased significantly ($P<0.05$) with time, from an initial level of 79.89% to 67.11 - 70.78%, while the lipid content of charr flesh increased substantially from 1.58% to 7.19 - 12.54% during the first 20 weeks of feeding experiment, and then decreased significantly ($P<0.05$) to 5.72 - 10.84%. The protein content increased as fish grew, but the mineral content remained relatively unchanged over the experimental period (Fig. 3.2). After 8 weeks on the experimental diets, the flesh moisture and lipid contents were significantly ($P<0.01$) influenced by the amount of dietary lipid, while the protein and mineral content of flesh were not ($P>0.05$) affected (Appendix 3). Regression analyses indicated that the flesh moisture was inversely related to dietary lipid content ($r=0.96$) and there was a significant direct relationship between the content of flesh lipid and that of dietary lipid ($r=0.96$).

The various dietary carotenoid levels did not affect the proximate composition of the flesh ($P>0.05$). No interaction of dietary lipid and carotenoids on the proximate composition of fish flesh was observed ($P>0.05$).

Table 3.2 Proximate composition (%) of the flesh of Arctic charr fed on different diets for 24 weeks[1]

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	$P<0.01$	$P<0.05$	$P<0.01$	$P>0.05$
Dietary carotenoid	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$
Interaction of dietary lipid and carotenoid	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$

[1]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, respectively.

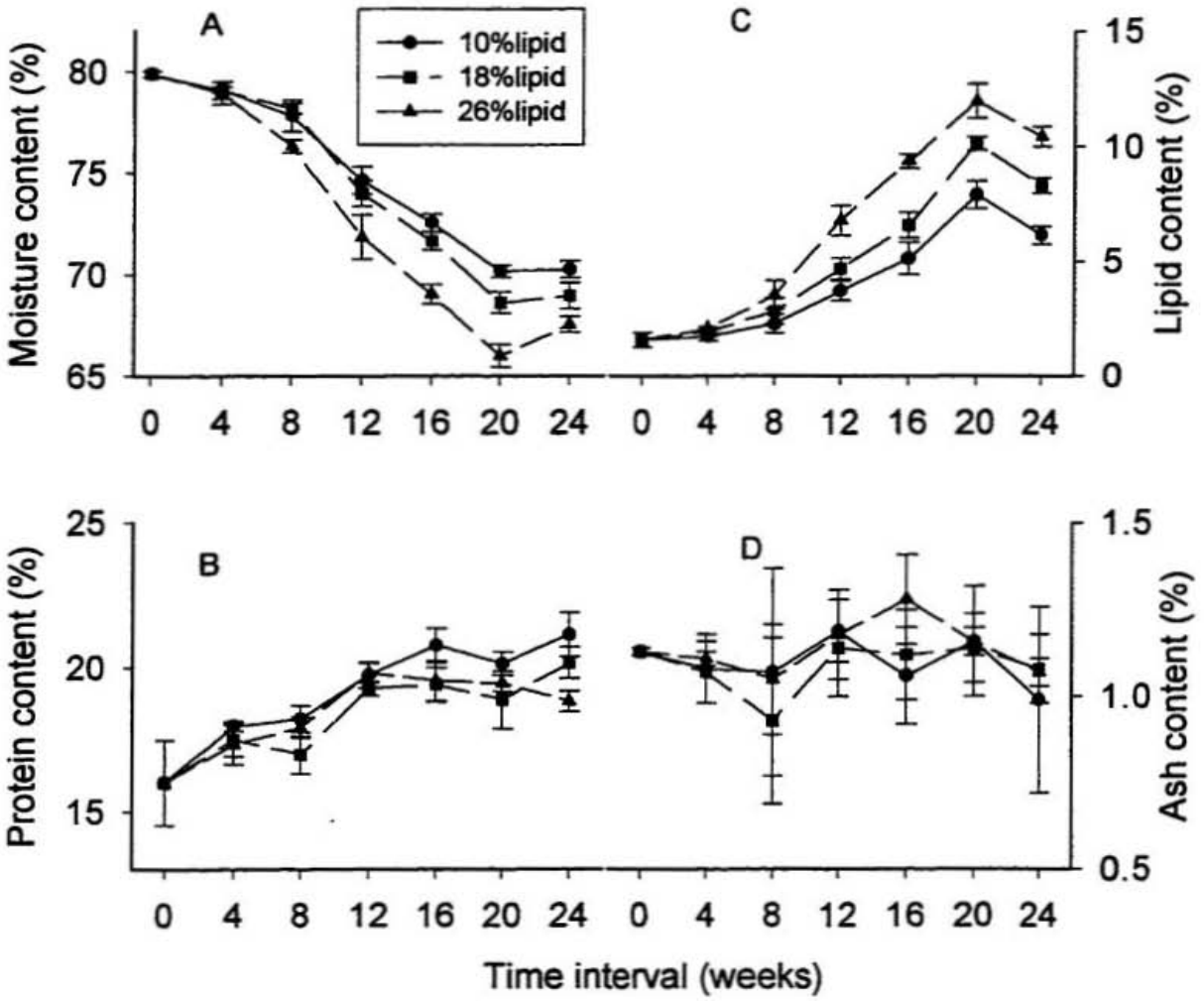

Fig. 3.2 Moisture content (A), crude protein (B), total lipid (C), and ash content (D) in the flesh of Arctic charr fed on different diets over a 24-week period

Note: Since no influence of dietary carotenoid, or any interaction of dietary lipid and carotenoid on the proximate composition were found, each curve above represents the mean value of groups at the same dietary lipid levels, respectively.

3.2.2 Lipid fatty acid composition of Charr flesh

The lipid fatty acid composition of flesh of Arctic charr prior to and after 24 weeks of feeding are given in Table 3.3. After feeding on experimental diets for 24 weeks, the content of saturated and monounsaturated fatty acids (**MUFA**) in the fish flesh increased significantly ($P<0.05$) from initial amounts of 16.42 and 32.74% to 22.5-24.49 and 50.48-53.41%, respectively. Meanwhile the amount of polyunsaturated fatty acids (**PUFA**) decreased significantly ($P<0.05$) from 50.86 to 23.77-25.72%. There was no significant ($P>0.05$) difference between the fatty acid composition of feed (Table 2.4) and those of fish flesh fed 18 and 26 % lipid, while the fish fed 10 % lipid contained higher **MUFA** and lower **PUFA** in their flesh as compared to that of the feed. Neither dietary lipid nor carotenoid levels had any effect on the fatty acid composition of fish flesh.

3.2.3 Liver lipid and hepatosomatic indices

The fish receiving no pigment supplement (i.e. the control group) had significantly ($P<0.05$) higher liver lipid content than those receiving dietary astaxanthin (Table 3.4). There was also a remarkable difference in hepatosomatic indices (**HSI**), the weight ratio of liver to the whole fish on a wet weight basis (Love, 1992), between the fish fed on pigmented diet and those on the control diet. The **HSI**

Table 3.3 Lipid fatty acid composition (%) of the flesh of Arctic charr prior to and after 24 weeks on experimental diets[1]

Fatty acid	Initial[2]	Diets (% lipid - ppm carotenoid)									
		18 - 0	10 - 40	10 - 60	10 - 80	18 - 40	18 - 60	18 - 80	26 - 40	26 - 60	26 - 80
12:0	-	0.07[a]	0.07[a]	0.07[a]	0.07[a]	0.06[a]	0.08[a]	0.12[a]	0.05[a]	0.05[a]	0.16[a]
14:0	2.05[b]	6.57[a]	5.04[a]	5.13[a]	5.2[a]	6.09[a]	6.15[a]	6.39[a]	6.65[a]	6.63[a]	6.72[a]
16:0	12.18[b]	15.46[a]	15.80[a]	15.72[a]	16.23[a]	15.43[a]	15.43[a]	15.81[a]	13.87[a]	14.21[a]	14.17[a]
18:0	2.19[a]	2.12[a]	2.97[a]	2.90[a]	2.79[a]	2.17[a]	2.14[a]	2.29[a]	1.81[a]	1.79[a]	1.94[a]
20:0	-	0.14[a]	0.14[a]	0.14[a]	0.14[a]	0.13[a]	-	0.13[a]	0.12[a]	0.11[a]	0.12[a]
Σ Sat.	16.42[b]	24.36[a]	24.02[a]	23.96[a]	24.43[a]	23.88[a]	23.80[a]	24.74[a]	22.5[a]	22.79[a]	23.11[a]
14:1ω5	-	-	0.12[a]	0.15[a]	0.15[a]	-	-	-	-	-	-
16:1ω7	4.09[b]	8.72[a]	9.03[a]	9.58[a]	9.55[a]	8.31[a]	8.25[a]	8.50[a]	7.68[a]	8.08[a]	7.95[a]
18:1ω9	10.08[b]	17.59[a]	19.85[a]	20.67[a]	19.96[a]	16.88[a]	17.03[a]	17.14[a]	16.71[a]	16.66[a]	16.28[a]
20:1ω9	10.93[b]	12.94[a]	11.01[a]	11.00[a]	10.92[a]	13.16[a]	13.20[a]	13.11[a]	15.10[a]	14.43[a]	15.05[a]
22:1ω11	7.64[b]	11.95[a]	7.18[a]	7.68[a]	7.38[a]	12.45[a]	12.00[a]	11.93[a]	13.22[a]	14.04[a]	13.59[a]
24:1ω9	-	0.19[a]	0.16[a]	0.17[a]	-	0.22[a]	-	-	0.21[a]	0.2[a]	0.22[a]
Σ Mono	32.74[b]	51.39[a]	47.35[a]	49.25[a]	47.96[a]	51.02[a]	50.48[a]	50.68[a]	52.92[a]	53.41[a]	53.09[a]
18:2ω6	3.28[a]	2.13[b]	3.09[b]	3.04[b]	3.08[b]	2.01[b]	1.98[b]	1.93[b]	1.84[b]	1.69[b]	1.67[b]
18:3ω6	-	0.08[a]	0.08[a]	0.09[a]	-	0.08[a]	-	-	0.08[a]	0.08[a]	-
18:3ω3	-	0.70[a]	0.63[a]	0.61[a]	0.61[a]	0.70[a]	0.73[a]	0.73[a]	0.84[a]	0.81[a]	0.83[a]
20:2ω6	-	0.21[a]	0.30[a]	0.29[a]	0.31[a]	0.26[a]	0.27[a]	0.25[a]	0.28[a]	0.26[a]	0.31[a]
20:3ω6	-	0.21[a]	0.20[a]	0.22[a]	0.23[a]	0.18[a]	-	-	0.16[a]	0.15[a]	-
20:3ω3	-	-	-	0.22[a]	-	0.11[a]	-	-	0.13[a]	0.12[a]	0.12[a]
20:4ω6	13.99[a]	0.56[b]	0.72[b]	0.67[b]	0.69[b]	0.60[b]	0.55[b]	0.54[b]	0.55[b]	0.53[b]	0.54[b]
20:5ω3	14.24[a]	6.12[b]	6.56[b]	6.00[b]	6.20[b]	6.29[b]	6.56[b]	6.38[b]	6.57[b]	6.41[b]	6.34[b]
22:5ω3	1.53[a]	1.30[a]	1.92[a]	1.72[a]	1.78[a]	1.63[a]	1.69[a]	1.59[a]	1.63[a]	1.57[a]	1.55[a]
22:6ω3	17.82[a]	13.54[b]	15.13[b]	13.91[b]	14.72[b]	13.25[b]	13.94[b]	13.15[b]	12.50[b]	12.15[b]	12.44[b]
Σ Poly	50.86[a]	24.85[b]	28.63[b]	26.77[b]	27.62[b]	25.11[b]	25.72[b]	24.57[b]	24.58[b]	23.77[b]	23.80[b]
U/S	5.09[a]	3.13[b]	3.16[b]	3.17[b]	3.09[b]	3.19[b]	3.20[b]	3.04[b]	3.44[b]	3.39[b]	3.33[b]
Σ ω6	17.27[a]	3.19[b]	4.39[b]	4.31[b]	4.31[b]	3.13[b]	2.80[b]	2.72[b]	2.91[b]	2.71[b]	2.52[b]
Σ ω3	33.59[a]	21.66[b]	24.24[b]	22.46[b]	23.31[b]	21.98[b]	22.92[b]	21.85[b]	21.67[b]	21.06[b]	21.28[b]
ω6/ω3	0.51[a]	0.15[b]	0.18[b]	0.19[b]	0.18[b]	0.14[b]	0.12[b]	0.12[b]	0.13[b]	0.13[b]	0.12[b]

[1]Results are mean values of duplicate determinations. Standard deviation ranged from 0.00 to 0.97. Values in each row with different superscript are significantly different ($P<0.05$) from one another, respectively. Sat. Mono and Poly are saturated, monounsaturated and polyunsaturated fatty acids, respectively. U/S are the ratio of unsaturated to saturated fatty acids.

[2]Initial=fish prior to the experiments

Table 3.4 Lipid content of liver and hepatosomatic index (HSI) of Arctic charr after 24 weeks on experimental diets

Diet (% lipid - ppm carotenoid)	Liver lipids (%)[1]	HSI (%)[2]
10 - 40	15.27 ± 1.34^{c}	1.86 ± 0.34^{b}
10 - 60	15.05 ± 1.37^{c}	1.82 ± 0.32^{b}
10 - 80	12.16 ± 1.65^{d}	1.63 ± 0.50^{b}
18 - 40	15.13 ± 1.12^{c}	1.56 ± 0.23^{b}
18 - 60	14.09 ± 1.73^{c}	1.79 ± 0.37^{b}
18 - 80	11.38 ± 1.98^{d}	1.76 ± 0.65^{b}
26 - 40	12.31 ± 1.00^{e}	1.73 ± 0.34^{b}
26 - 60	11.52 ± 1.93^{e}	1.77 ± 0.38^{b}
26 - 80	9.35 ± 1.37^{b}	1.59 ± 0.26^{b}
Control (18 - 0)	21.66 ± 0.21^{a}	2.27 ± 0.35^{b}
Dietary lipids	$P<0.01$	$P>0.05$
Dietary carotenoids	$P<0.01$	$P>0.05$
Interaction of lipid and carotenoid	$P>0.05$	$P>0.05$

[1,2]Results are mean values of triplicate and 8-10 determinations ± standard deviation, respectively. Data in each column with different superscript are statistically different ($P<0.05$) from one another, respectively.

of fish fed on pigmented diet was lower than those on the control diet (Table 3.4). Except for the control group (i.e. receiving no carotenoid supplement), the liver lipid content of fish on dietary carotenoids was inversely related to the dietary lipid level. There was a significant ($P<0.01$) influence of dietary lipid and carotenoids on the lipid content of fish liver. However, hepatosomatic indices were not affected by the content of dietary lipids or the carotenoid levels (Table 3.4). No interaction of dietary lipids and carotenoids was observed on the liver lipid and hepatosomatic indices.

3.3 Pigmentation

3.3.1 Total carotenoid concentration in flesh and belly skin

3.3.1.1 Carotenoid concentration in flesh

The total carotenoid contents in the flesh of Arctic charr after 24 weeks of feeding on experimental diets are summarized in Table 3.5. With the exception of the control group, which was devoid of any dietary carotenoids, the total content of flesh carotenoids increased throughout the experimental period for all treatments (Figs. 3.3 and 3.4, Appendix 4). After 24 weeks of feeding, the carotenoid levels in fish flesh from all experimental groups increased significantly ($P<0.05$), from an initial amount of 0.11 mg/kg to 4.06-8.35 mg/kg , on a wet weight basis; this exceeds 4 mg/kg level

Table 3.5 Total carotenoid content (mg/kg tissues) in the flesh and belly skin of Arctic charr fed on astaxanthin-pigmented diets for 24 weeks

Diet (% lipid - ppm carotenoid)	Flesh		Belly skin	
	on wet basis	on dry basis	on wet basis	on dry basis
At start:	0.11 ± 0.10^{b}	0.57 ± 0.11^{b}	0.22 ± 0.05^{b}	0.78 ± 0.19^{b}
After 24 weeks:				
18 - 0	0.30 ± 0.13^{b}	0.95 ± 0.11^{b}	0.46 ± 0.13^{b}	0.99 ± 0.11^{b}
10 - 40	4.06 ± 0.24^{c}	13.91 ± 0.27^{c}	13.18 ± 0.13^{c}	33.45 ± 0.26^{c}
10 - 60	4.93 ± 0.50^{cd}	16.46 ± 0.18^{e}	15.77 ± 0.12^{d}	37.99 ± 0.32^{d}
10 - 80	5.57 ± 0.21^{e}	18.58 ± 0.75^{g}	13.80 ± 0.16^{c}	42.16 ± 0.40^{e}
18 - 40	4.59 ± 0.17^{cd}	14.71 ± 0.58^{d}	16.10 ± 0.28^{d}	45.26 ± 0.80^{f}
18 - 60	5.93 ± 0.60^{e}	19.71 ± 0.52^{h}	25.37 ± 0.54^{f}	69.40 ± 2.24^{h}
18 - 80	7.61 ± 0.51^{f}	24.32 ± 0.17^{i}	30.92 ± 0.25^{h}	84.44 ± 0.67^{j}
26 - 40	5.54 ± 0.11^{e}	17.24 ± 0.37^{f}	20.38 ± 0.79^{e}	51.89 ± 0.51^{g}
26 - 60	8.14 ± 0.50^{af}	24.74 ± 0.16^{j}	26.38 ± 0.17^{g}	77.43 ± 1.13^{i}
26 - 80	8.35 ± 0.10^{a}	25.91 ± 0.34^{a}	32.47 ± 0.39^{a}	92.99 ± 0.20^{a}
Dietary lipid	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
Dietary carotenoid	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
Interaction of dietary lipid and carotenoid	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$

[1]Results are mean values of triplicate determinations ± standard deviation. Values in each column with different superscript (a-j) are significantly different ($P<0.05$) from one another, respectively.

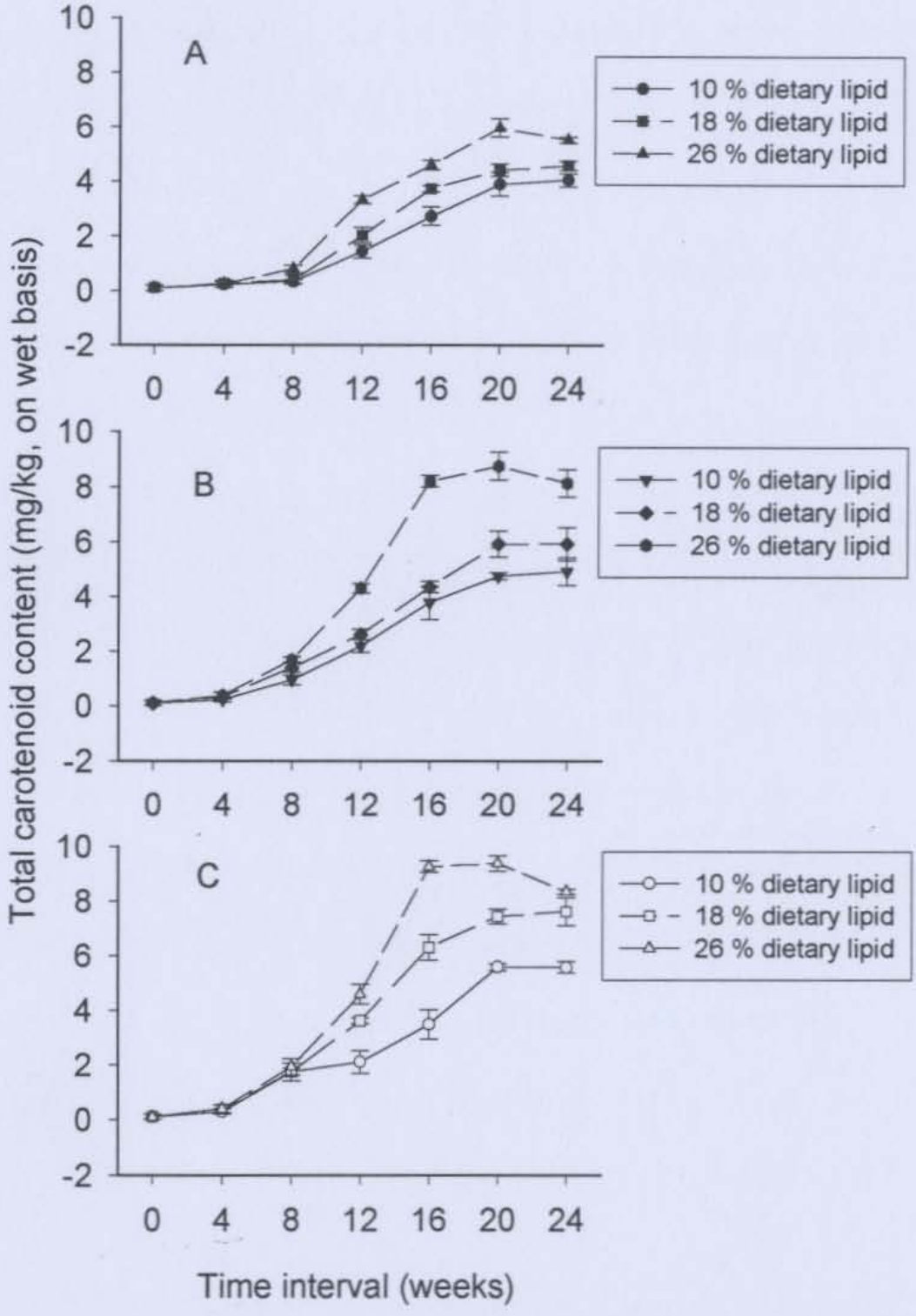

Fig. 3.3 Total carotenoid content (mg/kg, on wet-weight basis) in the flesh of Arctic charr fed experimental diets over a 24-week feeding period (I)

A, 40 ppm dietary carotenoid; B, 60 ppm dietary carotenoid;
C, 80 ppm dietary carotenoid

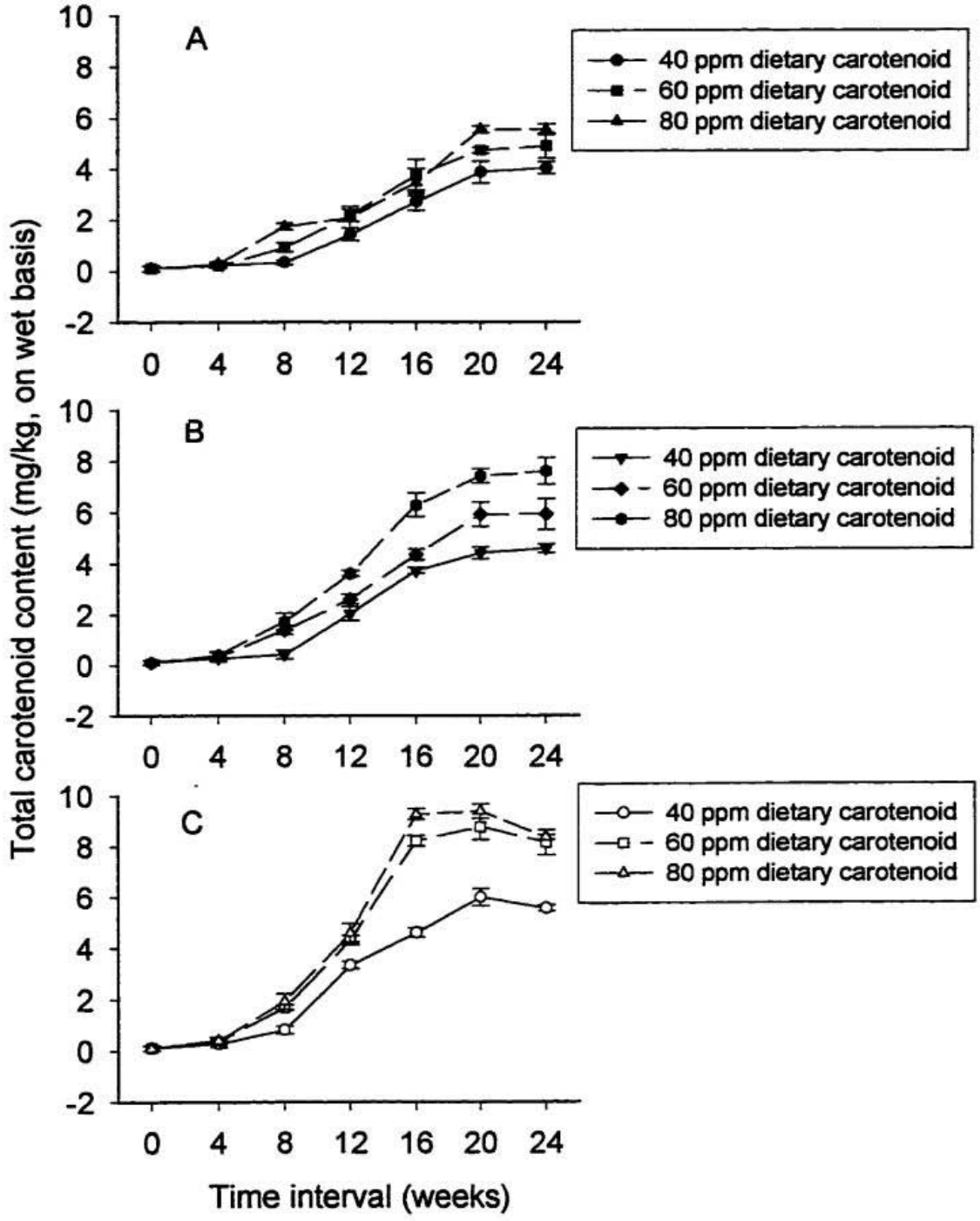

Fig. 3.4 Total carotenoid content (mg/kg, on wet-weight basis) in the flesh of Arctic charr fed experimental diets over a 24-week feeding period (II) A, 10% dietary lipid; B, 18% dietary lipid; C, 26% dietary lipid

which is generally regarded as sufficient for adequate visual colour impression of farmed salmon fish (Torrissen et al. 1989). For all treatments, the major changes (increase) in the pigment content of flesh were observed after 8 to 20 weeks of feeding. The flesh pigment content remained relatively unchanged after week-20 for the groups fed on diets containing 10 and 18 % dietary lipid, but decreased in the groups fed 26 % lipid in their feed (Fig. 3.4).

At each dietary carotenoid level (Fig. 3.3), after 8 weeks feeding, the total carotenoid content in fish flesh increased significantly ($P<0.05$) with increasing content of dietary lipids. The rate of increase in deposition of dietary carotenoids was the highest in the group receiving 26% dietary lipid, followed by the group fed 18% and 10% dietary lipid, respectively. At the 60 and 80 ppm dietary carotenoid levels (Fig. 3.3B and C), the carotenoid levels of fish flesh in the groups fed 26 % dietary lipid exceeded 4 mg/kg after 12 weeks of feeding, while fish in the groups receiving 10% dietary lipid reached the same carotenoid level of over 4 mg/kg after 20 weeks. For the groups on 40 ppm dietary carotenoids (Fig. 3.3A), the total pigment content in fish flesh exceeded 4 mg/kg on week-16, 20, and 24 for those receiving 26, 18 and 10% dietary lipid, respectively.

On the other hand, after feeding on formulated diets containing 18 % lipid for 8 weeks (Fig. 3.4B), the content of flesh pigments increased significantly ($P<0.05$)

as the amount of dietary carotenoids was increased. At the 26 % lipid level (Fig. 3.4C), there was a significant ($P<0.05$) increase in flesh carotenoid content when the amount of dietary carotenoids increased from 40 to 60 ppm, but the corresponding increase was less pronounced when the level of dietary carotenoids increased from 60 to 80 ppm. At the 10 % dietary lipid level, after 20 weeks of pigmentation, the difference in the carotenoid content of flesh among the three dietary pigment groups was slight but statistically significant ($P<0.05$), with the highest pigment content in the flesh of fish fed on 80 ppm dietary carotenoids, followed by the group receiving 60 and 40 ppm dietary carotenoids, respectively. There was a significant ($P<0.05$) interaction of dietary lipid and carotenoids on the total pigment content of fish flesh.

3.3.1.2 Total carotenoid content in belly skin

The total carotenoid contents in the belly skin of Arctic charr on week-24 of pigmentation are shown in Table 3.5. Similarly, the content of carotenoids in the belly skin of fish from all experimental groups increased during the course of the experiment (Figs. 3.5 and 3.6, Appendix 5). At the end of the experimental period, the level of carotenoids in belly skin increased significantly ($P<0.05$) from 0.22 mg/kg, on a wet weight basis, to 13.18-32.47 mg/kg (Table 3.5). The major deposition of dietary carotenoids in the belly skin was found after 8 weeks of feeding on pigmented diets. The pattern of the effect of dietary lipid on the carotenoid content in the belly

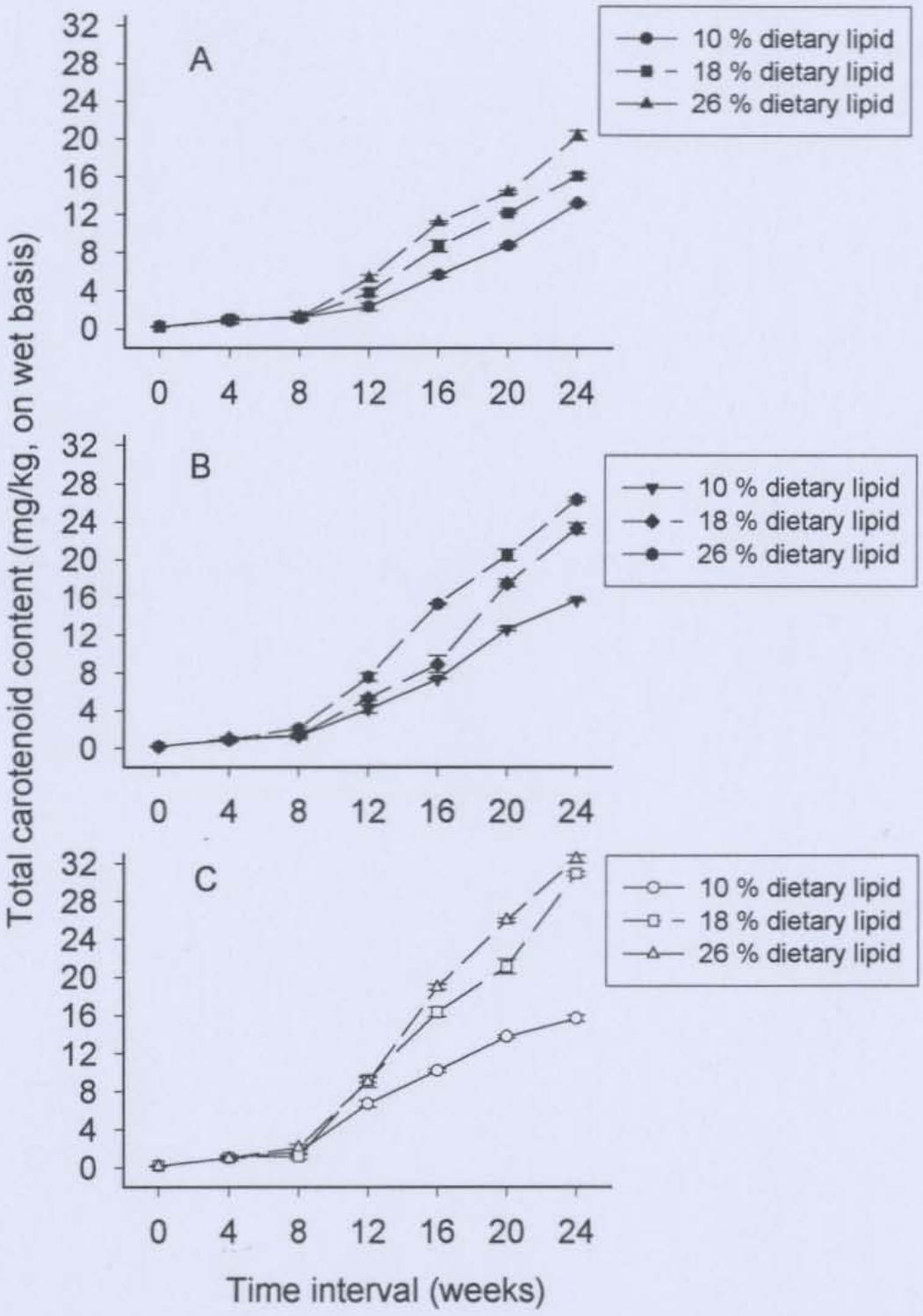

Fig. 3.5 Total carotenoid content (mg/kg, on wet-weight basis) in the belly skin of Arctic charr fed experimental diets over a 24-week feeding period (I)
A, 40 ppm dietary carotenoid; B, 60 ppm dietary carotenoid;
C, 8o ppm dietary carotenoid

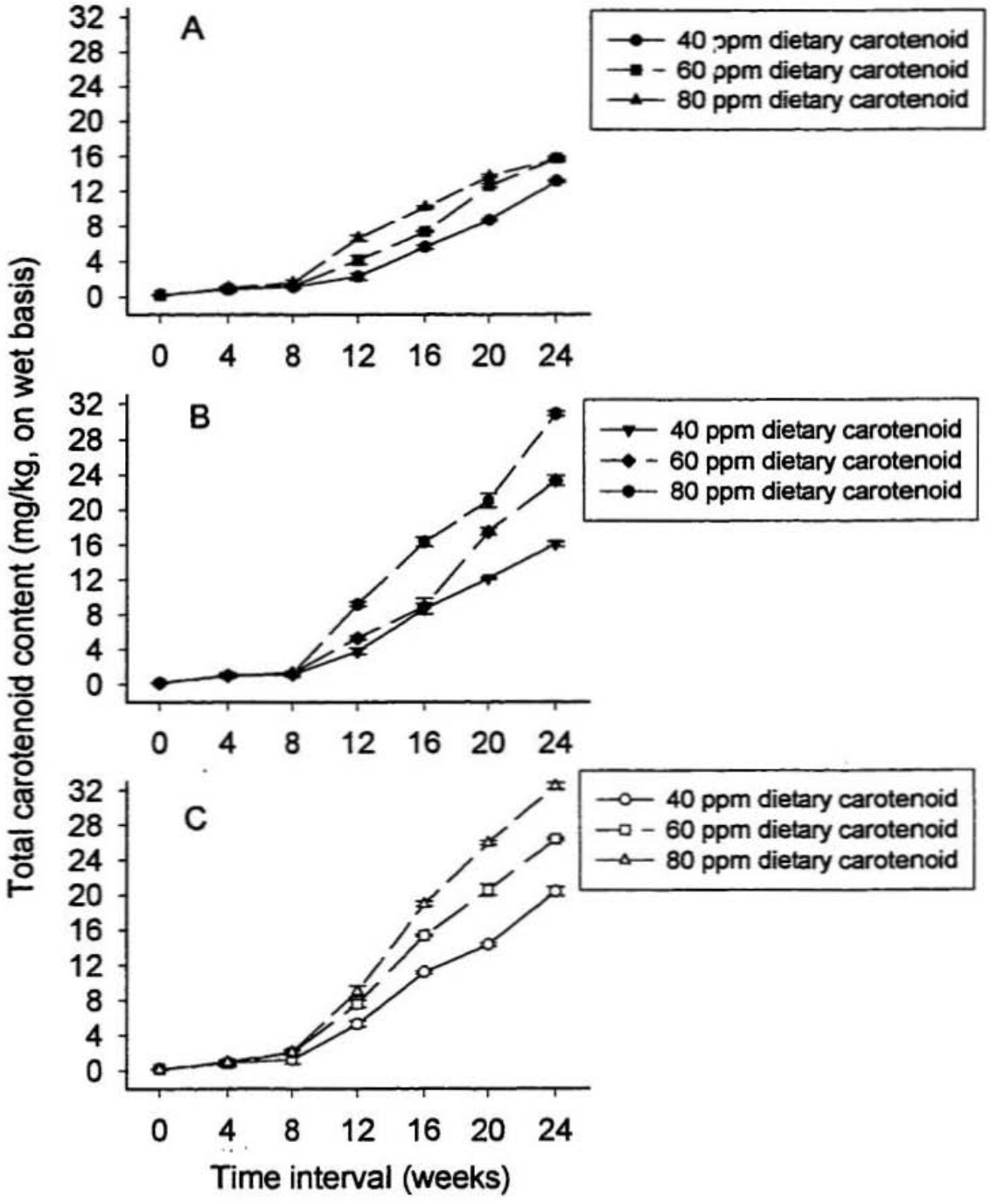

Fig. 3.6 Total carotenoid content (mg/kg, on wet-weight basis) in the belly skin of Arctic charr fed experimental diets over a 24-week feeding period (II) A, 10% dietary lipid; B, 18% dietary lipid; C, 26% dietary lipid

skin of fish was similar to that in their flesh. At each dietary carotenoid level, the pigment content of belly skin increased when the amount of dietary lipid was increased (Fig. 3.5). On the other hand, at 18 and 26 % (high) dietary lipid levels, the carotenoid contents in fish belly skin increased with the increase in the dietary carotenoid levels (Figs. 3.6B and C), whereas at the low dietary lipid level (i.e. 10 %), the increase in the amount of dietary carotenoids from 60 to 80 ppm did not result in any significant ($P<0.05$) difference in the pigment content of belly skin between the two treatments at the end of the experiment (Fig. 3.6A). A significant ($P<0.05$) interaction of dietary lipid and carotenoid on the pigment content of belly skin was also observed.

3.3.1.3 Correlation between flesh carotenoid concentration and fish size

Regression analysis indicated that there was a curvilinear relationship ($P<0.001$) between flesh pigmentation and fish body weight regardless of their levels of dietary carotenoid and lipid (Fig. 3.7). The regression equation is

$$y=-4.98+0.06x-0.00008x^2,\ r=0.88$$

where **y**=total carotenoid content (mg/kg, on wet basis) in flesh, **x**=body weight of fish (g).

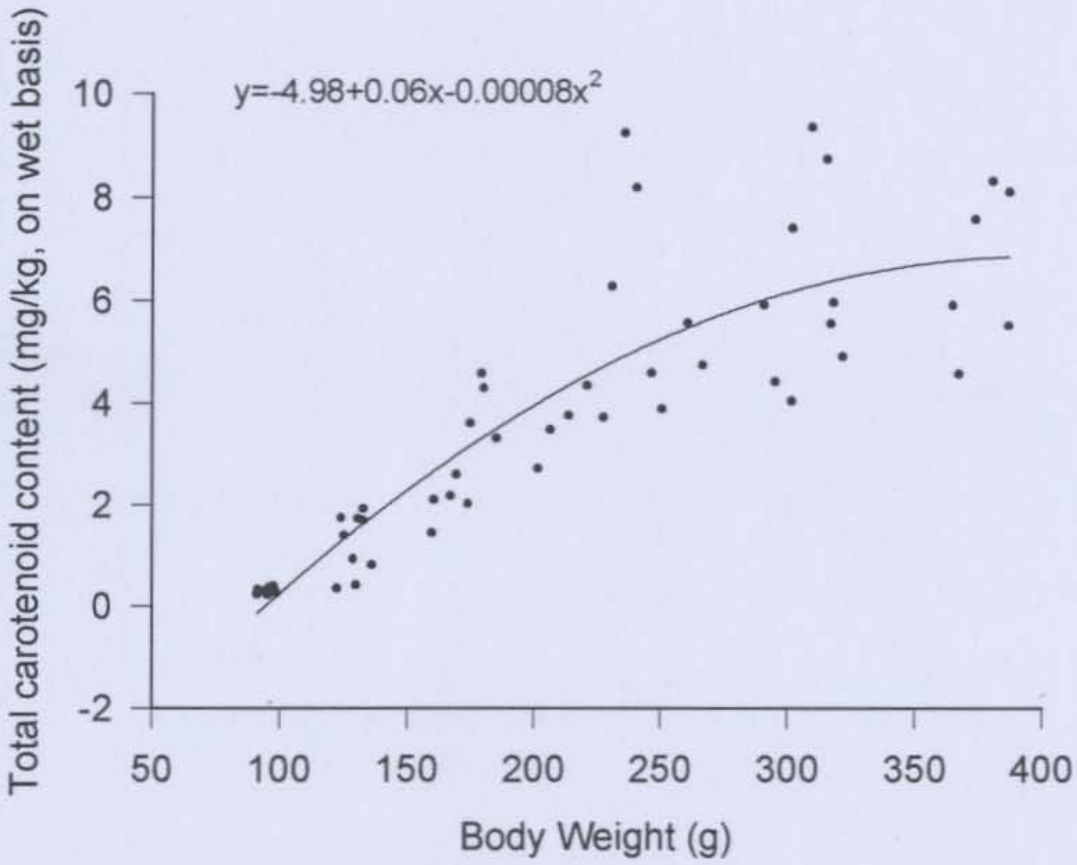

Fig. 3.7 The relation between total carotenoid content (mg/kg) of flesh and body weight of Arctic charr (n=54)

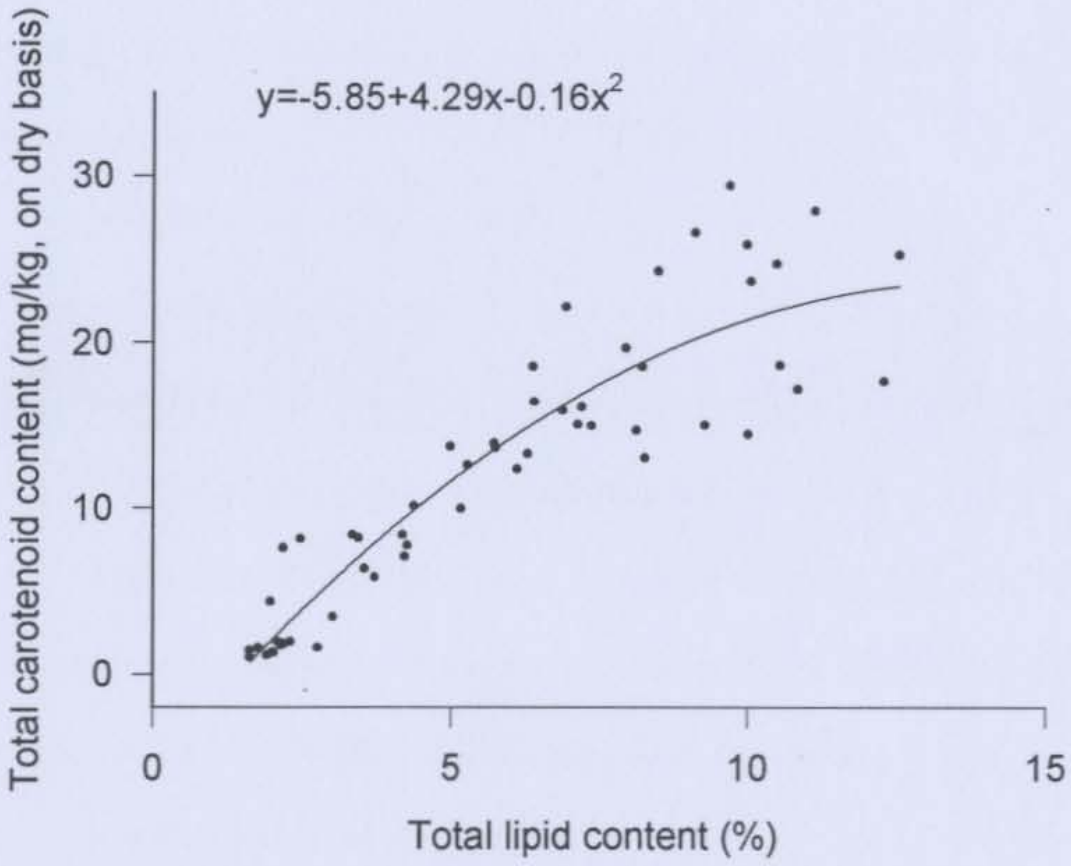

Fig. 3.8 The relationship between total carotenoid concentration (mg/kg) and lipid content (%) in the flesh of Arctic charr (n=60)

3.3.1.4 Correlation between carotenoid concentration and fat content in flesh

Regression analysis demonstrated that there was a high correlation ($P<0.0001$) between the total carotenoid and total fat contents in charr flesh (Fig. 3.8). The regression equation is

$$y=-5.85+4.29x-0.16x^2, \ r=0.92$$

where y=total carotenoid contents in flesh, x=total lipid levels in flesh.

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

The result of multiple linear regression revealed that the carotenoid concentration in charr flesh was directly correlated with both dietary lipid ($P=0.0022$) and carotenoid ($P=0.0008$) levels. The regression equation is

$$y=0.931+0.395x_1+0.191x_2, \ r=0.96$$

where y=total carotenoid content in flesh, x_1=dietary lipid levels, x_2=dietary carotenoid levels (Fig. 3.9). A similar relationship ($P=0.0033$) existed between carotenoid concentration in belly skin and dietary lipid and carotenoid levels, with a regression equation of

$$y=-28.3+1.85x_1+0.906x_2, \ r=0.92$$

where y=total carotenoid content in belly skin, x_1=dietary lipid levels, x_2=dietary

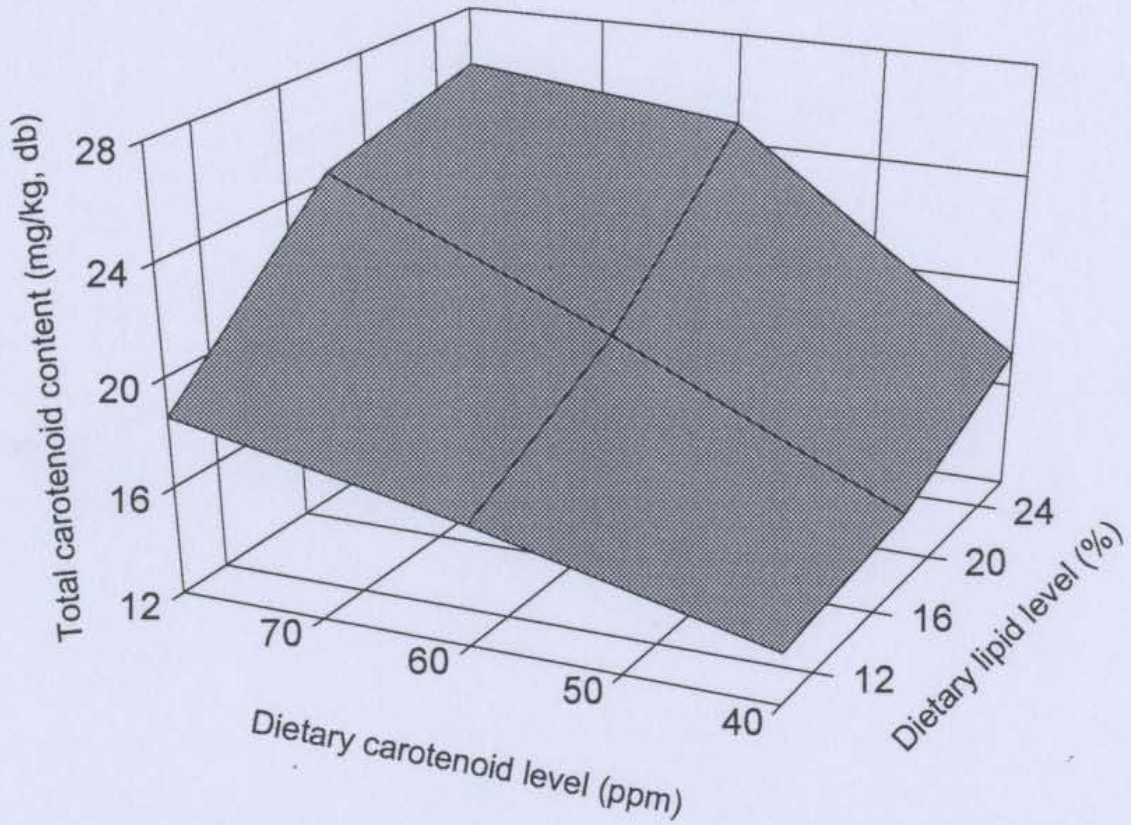

Fig. 3.9 The effects of dietary lipid (%) and carotenoid (ppm) on the total carotenoid contents (on dry-weight basis) in the flesh of Arctic charr.
$y=0.931+0.395x_1+0.191x_2$, where y=total carotenoid content in flesh, x_1=dietary lipid level, and x_2=dietary carotenoid level, n=9.

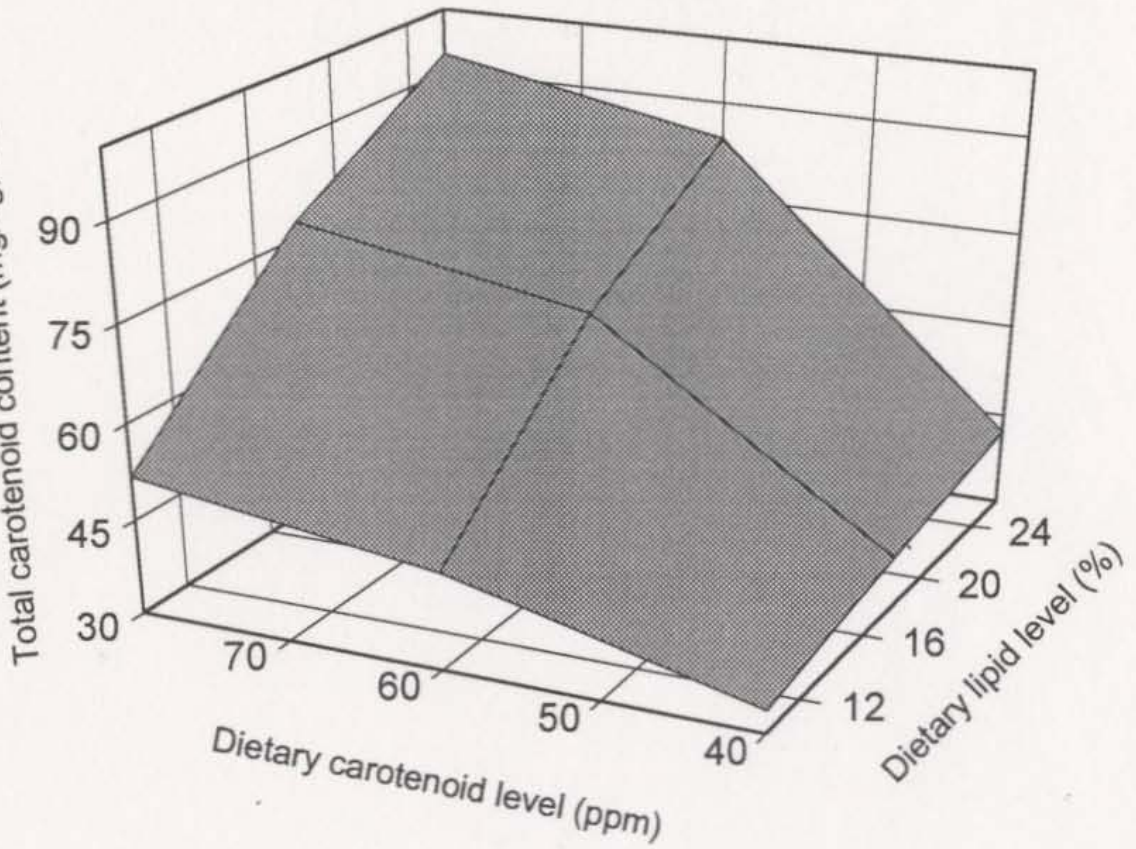

Fig. 3.10 The effects of dietary lipid (%) and carotenoid (ppm) on the total carotenoid contents (on dry-weight basis) in the belly skin of Arctic charr.

$y=-28.3+1.85x_1+0.906x_2$, where y=total carotenoid content in belly skin, x_1=dietary lipid level, x_2=dietary carotenoid levels, n=9.

carotenoid levels (Fig. 3.10).

3.3.2 Colour measurement

3.3.2.1 Colour parameters

The instrumentally measured colour values of filet, homogenized flesh and belly skin, of Arctic charr fed on experimental diets over a 24-week feeding period are given in Tables 3.6 to 3.8. The intensity of Hunter **a** (redness) and Hunter **b** (yellowness) values of filet, homogenized flesh and belly skin in all treatments (except for the control group) increased with progression of the duration of feeding on pigmented diets while Hunter **L** (lightness) values decreased (Figs. 3.11 to 3.16, Appendices 6 to 8).

Figs. 3.11 and 3.12 show a major increase in Hunter **a** value of filet beginning from week-8 to week-16 of feeding on pigmented diets for each experimental group. Prolonged feeding on carotenoid-containing diets did not result in any statistically significant ($P<0.05$) changes in Hunter colour values. At the end of the experiment (i.e. on week-24), at 80 ppm dietary carotenoid level, filet from the group fed on 26% dietary lipid and 80 ppm carotenoids exhibited the highest Hunter **a** value, followed by the group fed on 18 and 10 % dietary lipids (Fig. 3.11C). For fish receiving 40 and 60 ppm dietary pigments, the Hunter **a** values of filet were not significantly ($P>0.05$)

Table 3.6 Hunter L, a, b colour values of the filet of Arctic charr after 24 weeks of feeding on experimental diets[1]

Diet (% lipid - ppm carotenoid)	Hunter **L**	Hunter **a**	Hunter **b**
At start:	50.38 ± 1.74^{a}	0.28 ± 0.02^{b}	12.33 ± 1.07^{b}
After 24 weeks:			
18 - 0	49.69 ± 2.06^{a}	1.21 ± 0.86^{b}	12.39 ± 0.82^{b}
10 - 40	44.79 ± 1.51^{a}	15.14 ± 1.01^{c}	21.61 ± 1.30^{c}
10 - 60	43.18 ± 2.59^{a}	16.04 ± 1.33^{ac}	22.16 ± 1.57^{cd}
10 - 80	39.40 ± 1.10^{a}	17.25 ± 1.20^{ac}	23.09 ± 1.03^{ac}
18 - 40	41.62 ± 0.52^{a}	16.86 ± 1.80^{ac}	23.05 ± 1.93^{ac}
18 - 60	40.27 ± 0.97^{a}	18.37 ± 1.25^{ac}	23.99 ± 0.93^{ac}
18 - 80	41.61 ± 1.70^{a}	19.52 ± 2.83^{ac}	25.03 ± 1.30^{ac}
26 - 40	43.54 ± 3.14^{a}	17.56 ± 2.09^{ac}	23.86 ± 1.86^{ac}
26 - 60	42.19 ± 2.13^{a}	18.73 ± 1.76^{ac}	25.90 ± 1.17^{ad}
26 - 80	41.80 ± 2.43^{a}	20.49 ± 2.31^{a}	26.16 ± 1.46^{a}
Dietary lipid	$P>0.05$	$P<0.05$	$P<0.01$
Dietary carotenoid	$P>0.05$	$P<0.05$	$P<0.05$
Interaction of dietary lipid and carotenoid	$P>0.05$	$P>0.05$	$P>0.05$

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

Table 3.7 Hunter L, a, b colour values of the homogenized flesh of Arctic charr after 24 weeks of feeding on experimental diets[1]

Diet (% lipid - ppm carotenoid)	Hunter **L**	Hunter **a**	Hunter **b**
At start:	70.69 ± 1.15^{a}	0.08 ± 0.38^{b}	12.18 ± 0.24^{b}
After 24 weeks:			
18 - 0	65.33 ± 2.00^{f}	0.49 ± 0.40^{b}	13.24 ± 0.69^{b}
10 - 40	58.96 ± 0.50^{e}	10.50 ± 0.61^{c}	20.95 ± 0.61^{c}
10 - 60	58.30 ± 0.44^{ec}	11.71 ± 0.12^{d}	22.70 ± 1.31^{c}
10 - 80	55.07 ± 0.67^{b}	12.32 ± 0.44^{de}	23.70 ± 0.85^{c}
18 - 40	57.33 ± 0.67^{c}	12.51 ± 0.27^{e}	22.69 ± 0.39^{c}
18 - 60	56.03 ± 0.66^{bd}	14.01 ± 0.33^{f}	24.68 ± 1.17^{ac}
18 - 80	55.20 ± 0.45^{b}	14.36 ± 0.68^{f}	25.06 ± 0.95^{ac}
26 - 40	57.59 ± 0.37^{c}	13.26 ± 0.97^{fe}	23.09 ± 0.52^{ac}
26 - 60	57.54 ± 0.55^{c}	14.40 ± 0.54^{f}	25.44 ± 0.29^{ac}
26 - 80	56.74 ± 0.64^{cd}	15.98 ± 0.48^{a}	25.95 ± 0.43^{a}
Dietary lipid	$P<0.01$	$P<0.01$	$P<0.01$
Dietary carotenoid	$P<0.01$	$P<0.01$	$P<0.01$
Interaction of dietary lipid and carotenoid	$P<0.01$	$P<0.01$	$P>0.05$

[1]Results are mean values of 48 determinations ± standard deviation. Data in each column with different superscript are significant different ($P<0.05$), respectively.

Table 3.8 Hunter L, a, b colour values of the belly skin of Arctic charr after 24 weeks of feeding on experimental diets[1]

Diet (% lipid - ppm carotenoid)	Hunter L	Hunter a	Hunter b
At start:	81.06 ± 1.58^{a}	-1.46 ± 0.28^{b}	5.40 ± 1.42^{b}
After 24 weeks:			
18 - 0	72.05 ± 1.84^{a}	-0.51 ± 0.98^{b}	11.52 ± 2.29^{b}
10 - 40	67.27 ± 5.66^{a}	9.12 ± 2.44^{c}	12.21 ± 1.04^{c}
10 - 60	62.40 ± 2.57^{a}	12.34 ± 2.49^{ac}	14.31 ± 1.54^{ac}
10 - 80	63.96 ± 3.99^{a}	12.11 ± 2.60^{ac}	15.52 ± 0.81^{ac}
18 - 40	66.23 ± 2.58^{a}	9.63 ± 3.92^{c}	13.07 ± 1.05^{ac}
18 - 60	65.15 ± 3.84^{a}	11.90 ± 2.26^{ac}	16.82 ± 1.91^{ac}
18 - 80	62.00 ± 6.37^{a}	16.62 ± 3.57^{ac}	18.70 ± 1.14^{a}
26 - 40	65.84 ± 3.77^{a}	12.46 ± 2.52^{ac}	14.68 ± 1.53^{ac}
26 - 60	63.54 ± 5.57^{a}	14.72 ± 2.60^{ac}	17.43 ± 2.91^{ac}
26 - 80	62.86 ± 5.44^{a}	19.44 ± 4.69^{a}	19.10 ± 2.27^{a}
Dietary lipid	$P>0.05$	$P<0.05$	$P<0.05$
Dietary carotenoid	$P>0.05$	$P<0.01$	$P<0.01$
Interaction of dietary lipid and carotenoid	$P>0.05$	$P>0.05$	$P>0.05$

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

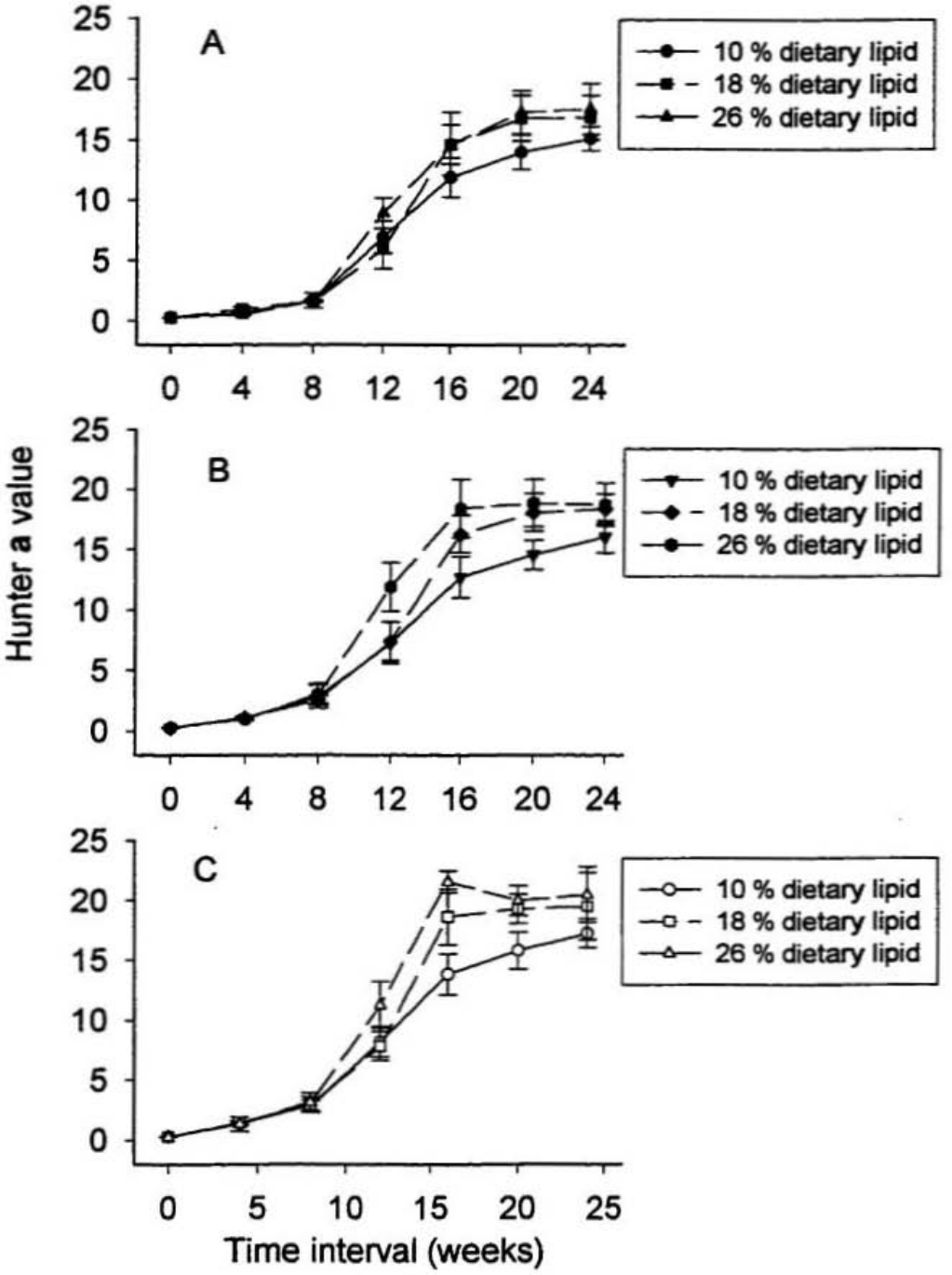

Fig. 3.11 Hunter-Colormet color scale **a** values of the filet of Arctic charr fed experimental diets over a 24-week feeding period (I)

A, 40 ppm dietary carotenoid; B, 60 ppm dietary carotenoid; C, 80 ppm dietary carotenoid

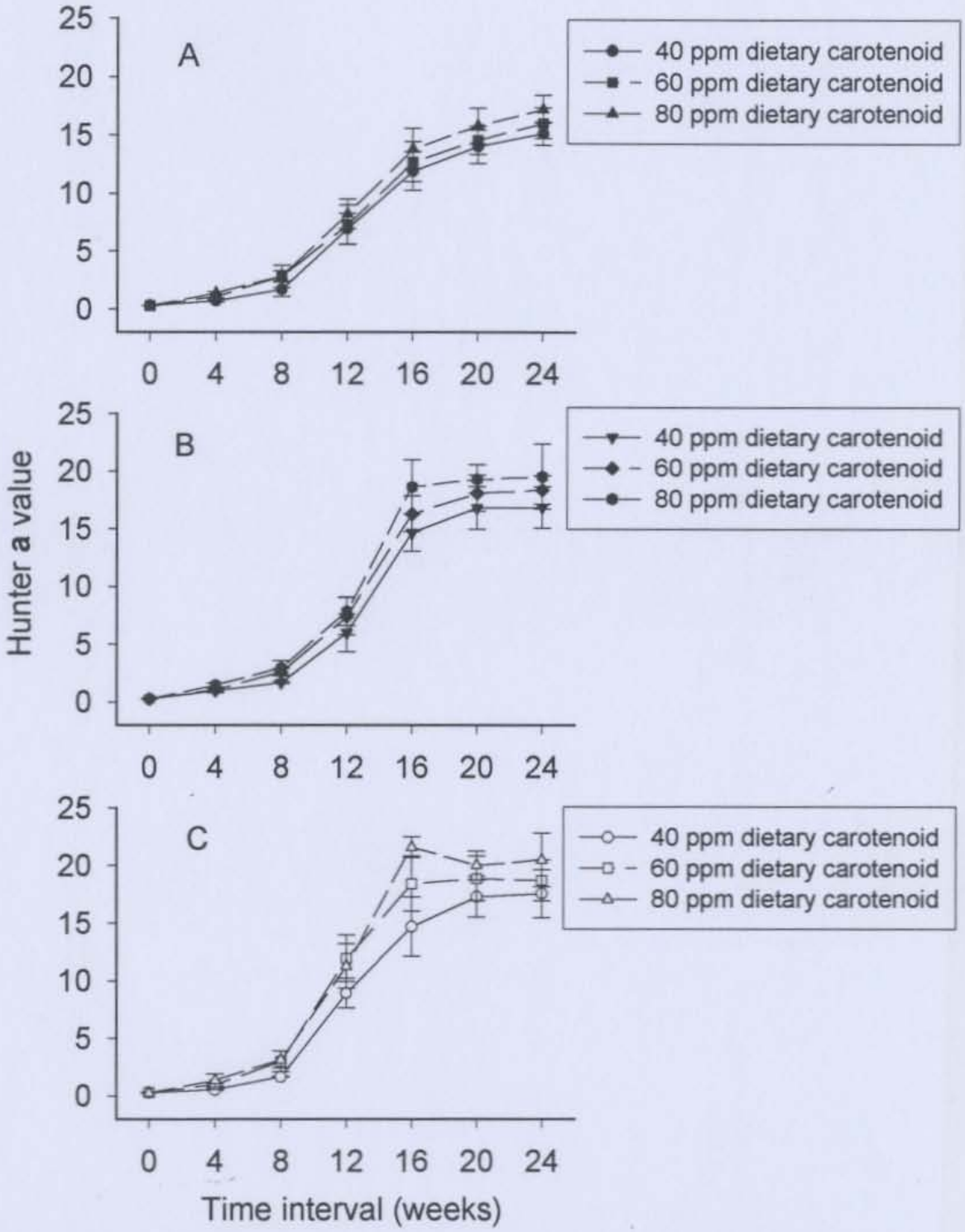

Fig. 3.12 Hunter-Colormet color scale **a** values of the filet of Arctic charr fed experimental diets over a 24-week feeding period (II)

A, 10% dietary lipid; B, 18% dietary lipid; C, 26% dietary lipid

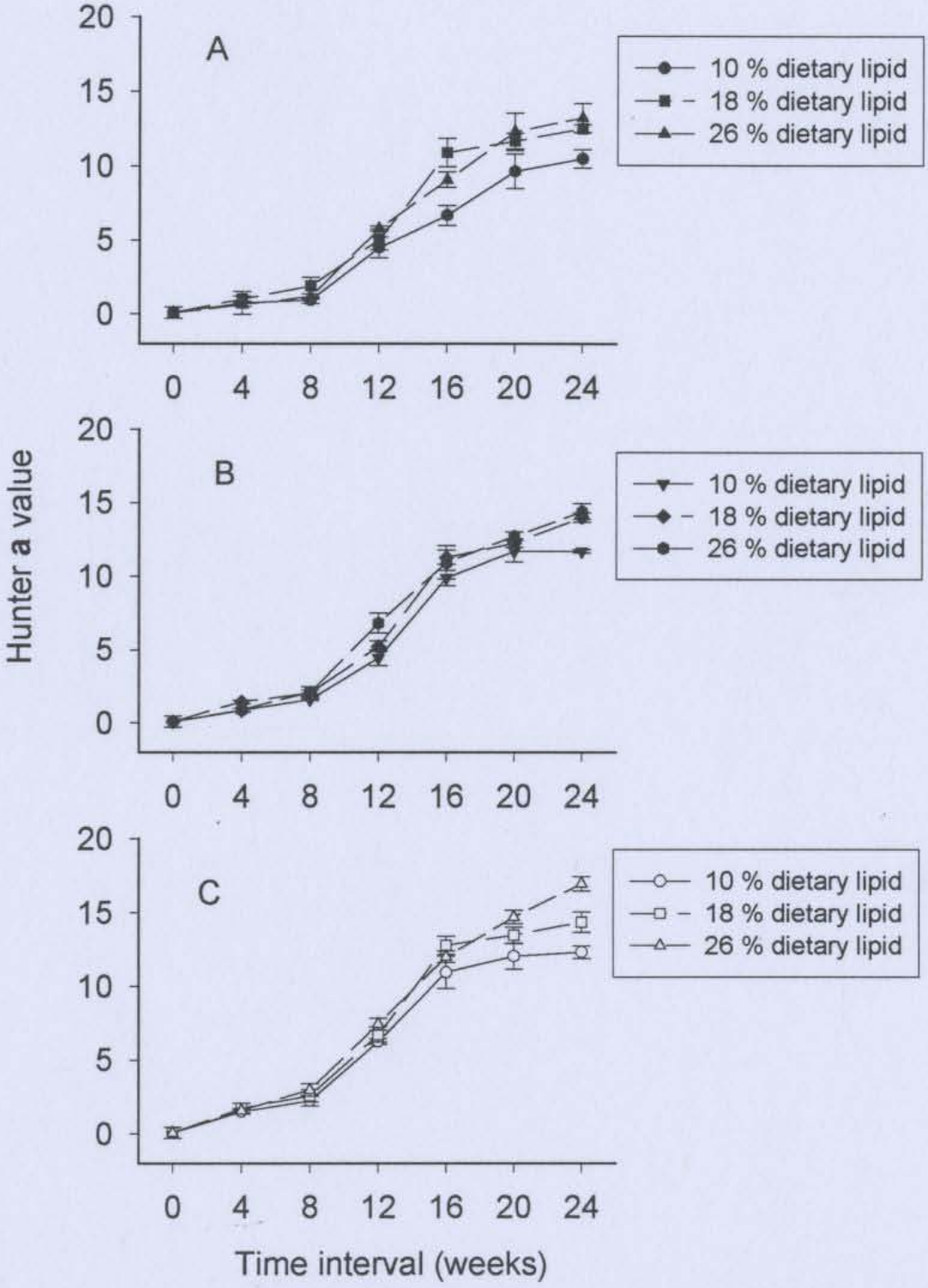

Fig. 3.13 Hunter-Colormet color scale **a** values of the flesh of Arcitc charr fed experimental diets over a 24-week feeding period (I)

A, 40 ppm dietary carotenoid; B, 60 ppm dietary carotenoid; C, 80 ppm dietary carotenoid

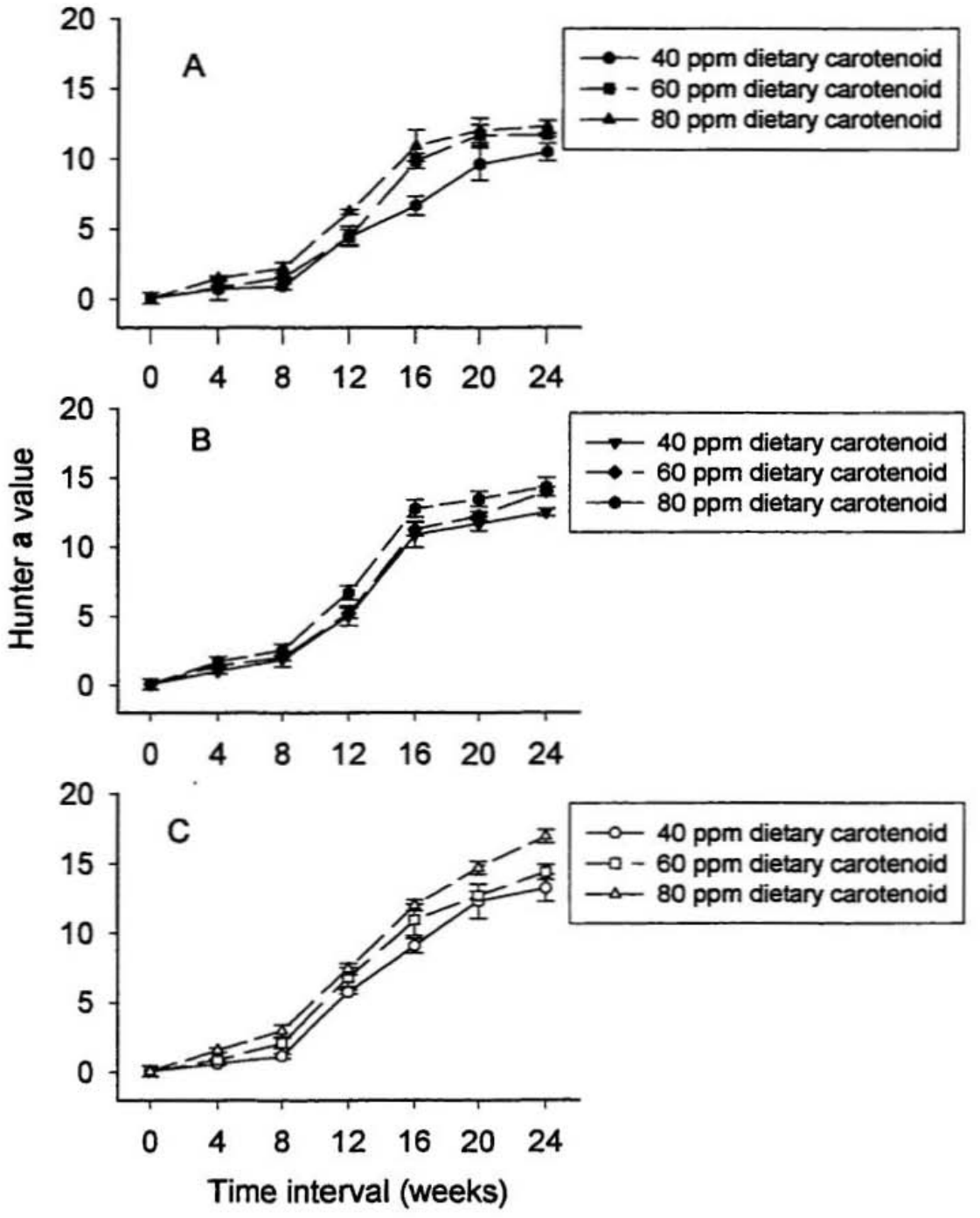

Fig. 3.14 Hunter-Colormet color scale a value of the flesh of Arctic charr fed experimental diets over a 24-week feeding period (II)
A, 10% dietary lipid; B, 18% dietary lipid; 26% dietary lipid

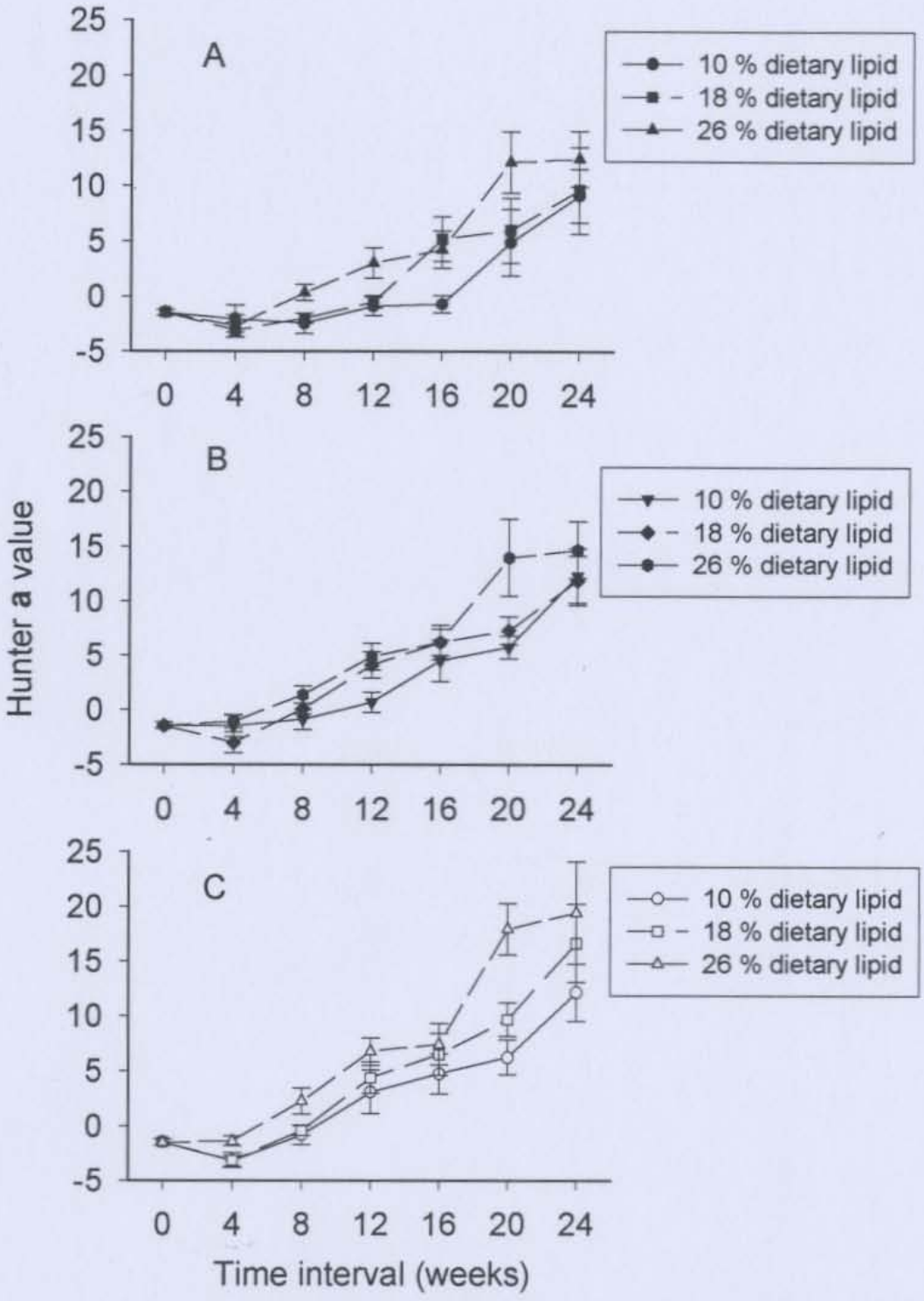

Fig. 3.15 Hunter-Colormet color scale **a** values of the belly skin of Arctic charr fed experimental diets over a 24-week feeding period (I)
A, 40 ppm dietary carotenoid; B, 60 ppm dietary carotenoid;
C, 80 ppm dietary carotenoid

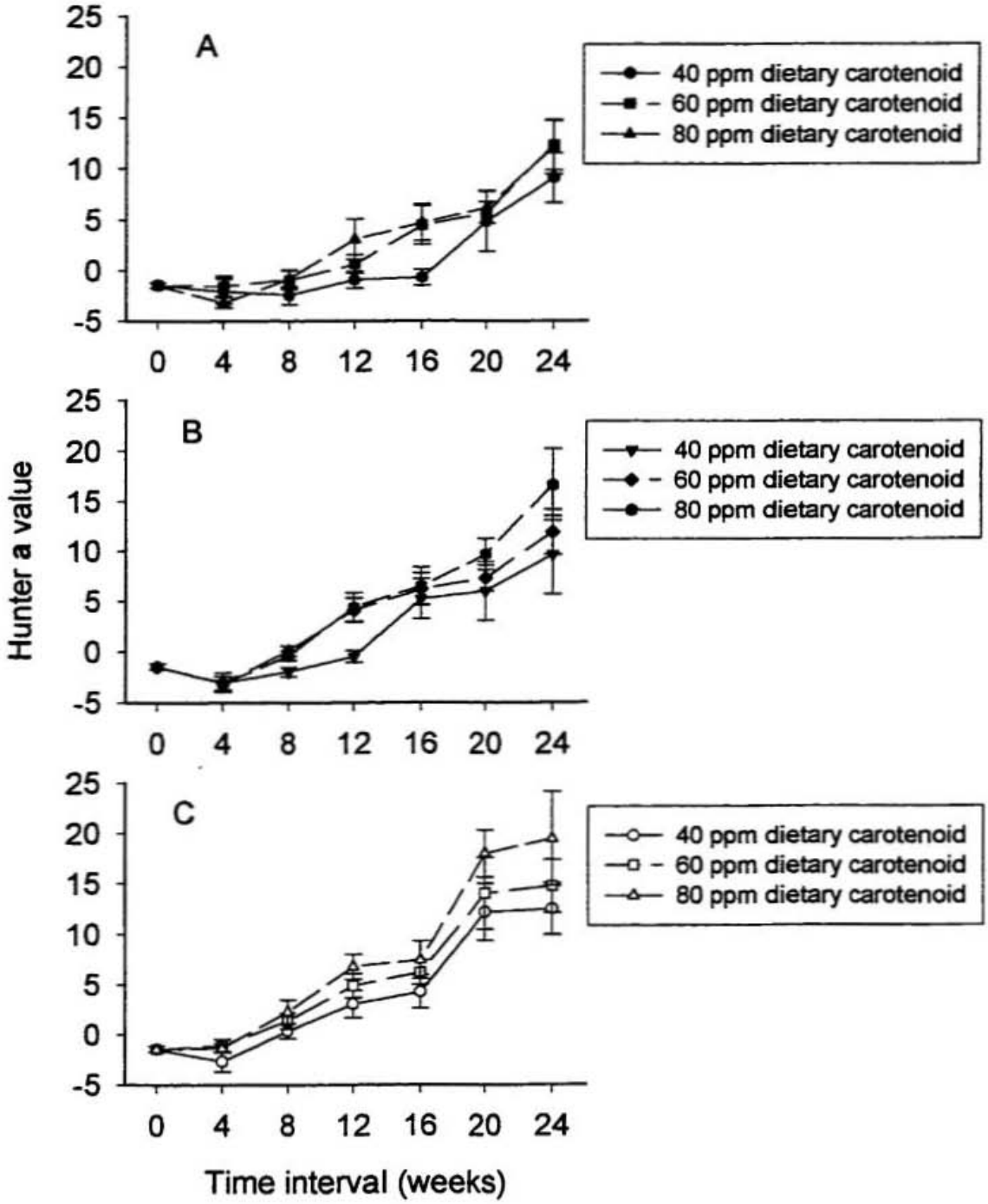

Fig. 3.16 Hunter-Colormet color scale a values of the belly skin of Arctic charr fed experimental diets over a 24-week feeding period (II)
A, 10% dietary lipid; B, 18% dietary lipid; C, 26% dietary lipid

different from those receiving 18 and 26 % dietary lipids (Figs. 3.11A and 3.11B). Furthermore, groups receiving 18 and 26 % dietary lipids and 80 ppm carotenoids exhibited the highest Hunter **a** values of filet after 24 weeks pigmentation, followed by the group receiving 60 and 40 ppm dietary carotenoid (Figs. 3.12B and 3.12C). An increase in the dietary carotenoid level from 40 to 60 ppm did not result in any significant ($P>0.05$) increase in the Hunter **a** value of filet when fish received low levels of dietary lipid (i.e. 10%) (Fig. 3.12A). A similar pattern was also observed for homogenized charr flesh (Figs. 3.13 and 3.14).

There was a significant ($P<0.01$) interaction of dietary lipid and carotenoid levels on Hunter **L, a** values of homogenized flesh, but not on the Hunter **b** value of flesh (Table 3.7, Appendix 8).

After 16 weeks of feeding on pigmented diets at different levels, the Hunter **a** value of belly skin in the group fed on 26% dietary lipid was significantly higher than those receiving 10 and 18% dietary lipid (Fig 3.15). The major change in the Hunter **a** value of belly skin of the group receiving 26 % dietary lipid was achieved during the first 20 weeks of the experiment. After 20 weeks, the Hunter **a** value in the same groups remained relatively unchanged while the Hunter **a** values of the belly skin of groups receiving 18 and 10 % dietary lipid continued to increase significantly ($P<0.05$). On the other hand, at 18 and 26% dietary lipid level, the belly skin of fish

fed 80 ppm dietary pigment attained the highest Hunter **a** value, followed by those receiving 60 and 40 ppm dietary carotenoids (Figs. 3.16B and C). For belly skin of fish on 10 % dietary lipid, no significant ($P>0.05$) increase in the Hunter **a** value was observed when the level of dietary carotenoids increased from 60 ppm to 80 ppm (Fig. 3.16A). No significant ($P>0.05$) interaction of dietary lipid and carotenoid levels was observed.

3.3.2.2 Correlation between colour values and carotenoid concentration in the flesh and belly skin of charr

The regression analysis on the Hunter colour values and pigment contents of the flesh and belly skin of charr, including the initial and control samples, indicated that the carotenoid concentration had a significant ($P<0.0001$) effect on colour variations as reflected in Hunter **L**, **a**, **b**.

As shown in Fig. 3.17, the intensity of the red colour of fish flesh, as reflected in their Hunter **a** value, increased in a curvilinear manner with increasing carotenoid levels in the flesh as given by the regression equation:

$$y=-0.76+0.84x-0.009x^2$$

where **y**= Hunter **a** value, and **x**=total carotenoid content in flesh (mg/kg, on dry basis). The **r** value for this regression equation was 0.96. The yellowness intensity of flesh, as noted in Hunter **b** value, also increased as carotenoid levels in the flesh

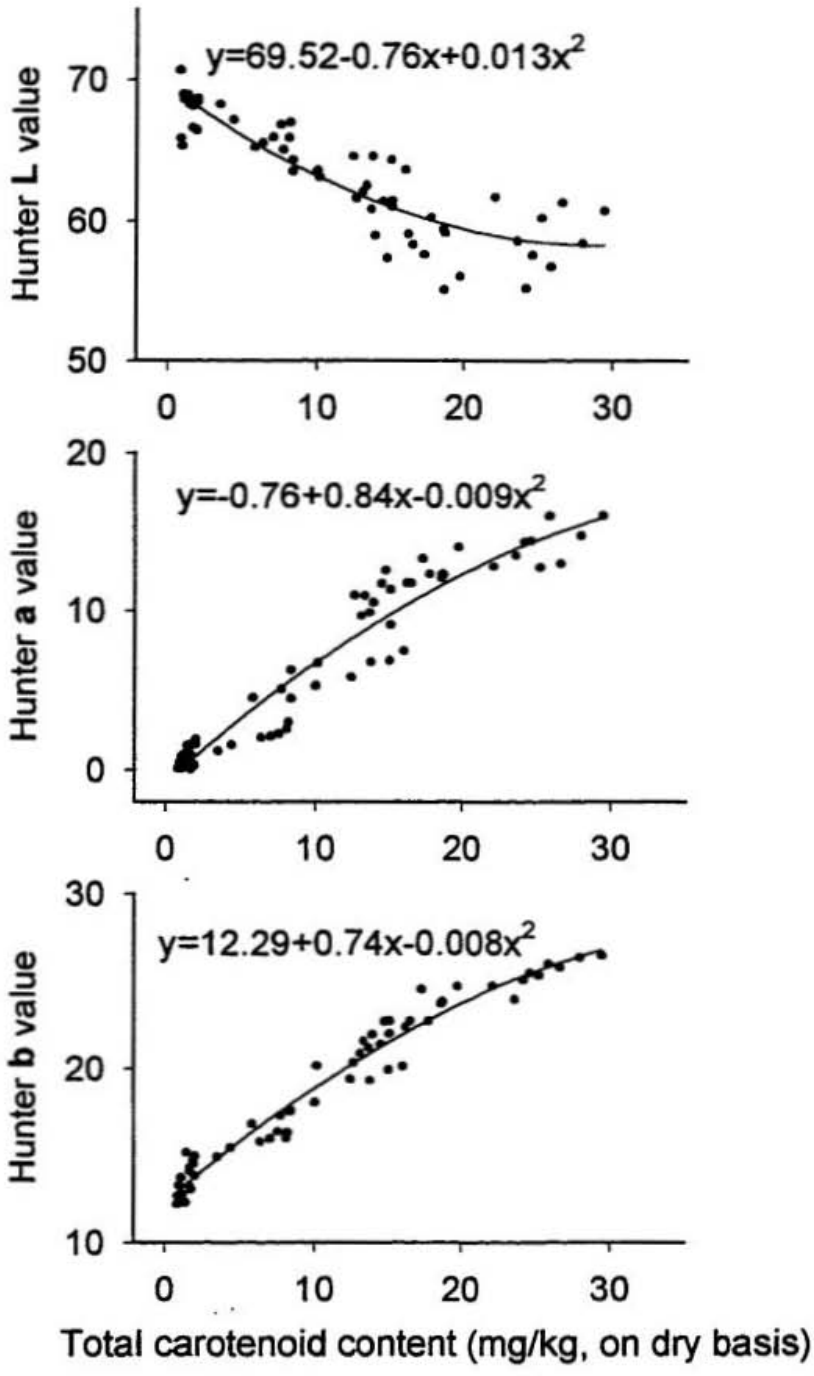

Fig. 3.17 The correlation between the Hunter **L**, **a**, and **b** values of homogenized flesh and total carotenoid levels in the flesh of Arctic charr (n=60)

increased, and a curvilinear relationship was found, as given below:

$$y=12.29+0.74x-0.008x^2$$

where **y**=Hunter **b** value, and **x** is total carotenoid levels (mg/kg, on dry basis) in the flesh. The **r** value was 0.98. However, an inverse relationship existed between lightness of flesh, as shown in Hunter **L** value, and carotenoid levels in the flesh of fish. The regression equation for this relationship is as follows:

$$y=69.52-0.76x+0.013x^2 \text{ , } r=0.90$$

where **y**=Hunter **L** value, **x**=total carotenoid content in flesh.

Similar pattens were also found for the relationship of the Hunter colour values and carotenoid levels in the filet and belly skin (Fig. 3.18 and 3.19). The regression equations are as follows:

For filet:

$y=-1.25+1.22x-0.015x^2$, r=0.96, (Hunter **a** value);

$y=11.37+0.75x-0.006x^2$, r=0.96, (Hunter **b** value);

$y=48.46-0.49x+0.008x^2$, r=0.88, (Hunter **L** value). (Fig. 3.18)

For belly skin:

$y=-2.33+0.38x-0.002x^2$, r=0.96, (Hunter **a** value);

$y=7.73+0.24x-0.001x^2$, r=0.89, (Hunter **b** value);

$y=78.04-0.34x+0.002x^2$, r =0.89, (Hunter **L** value). (Fig. 3.19)

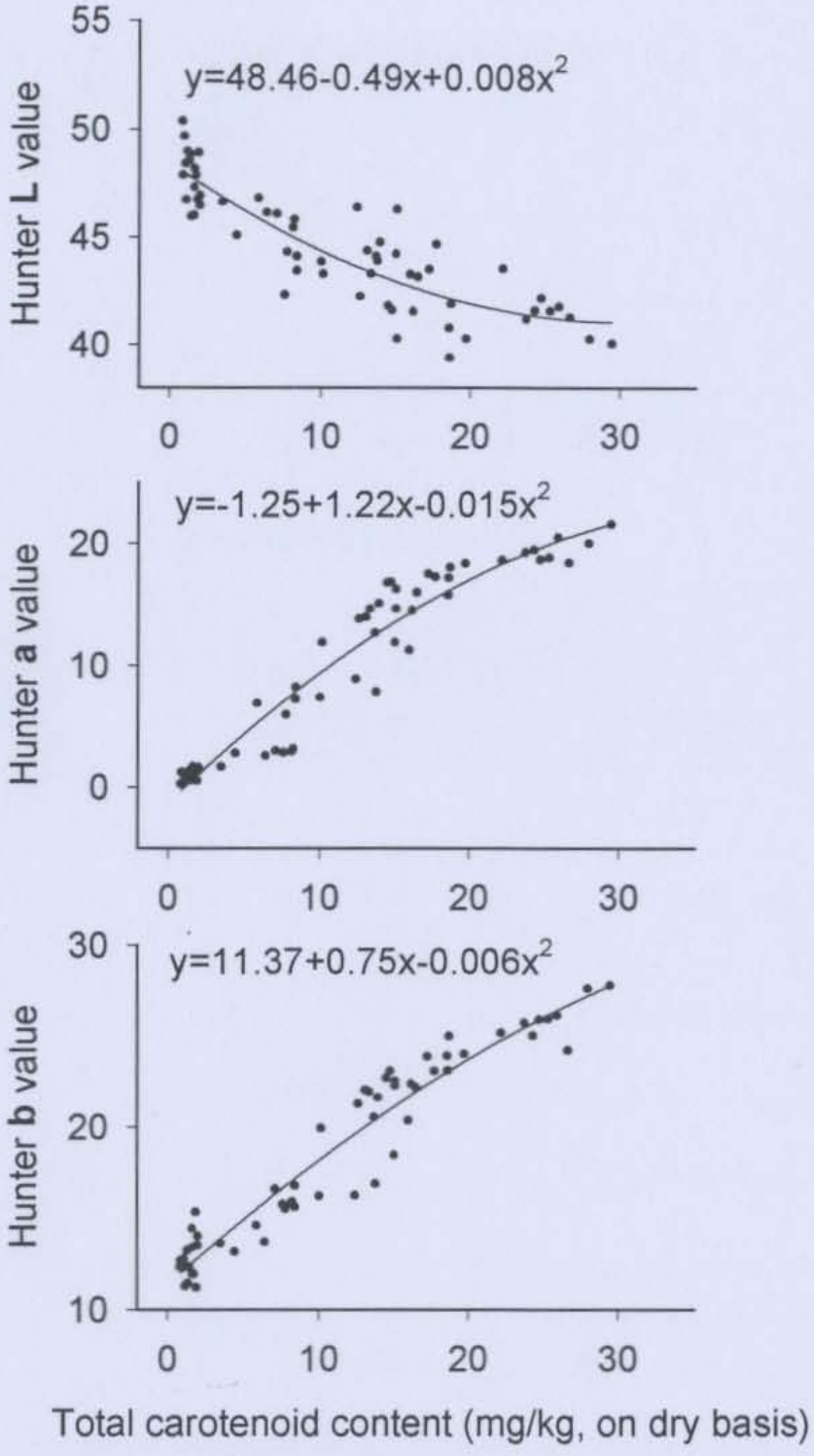

Fig. 3.18 The correlation between Hunter **L**, **a**, and **b** values of filet and total carotenoid levels in the flesh of Arctic charr (n=60)

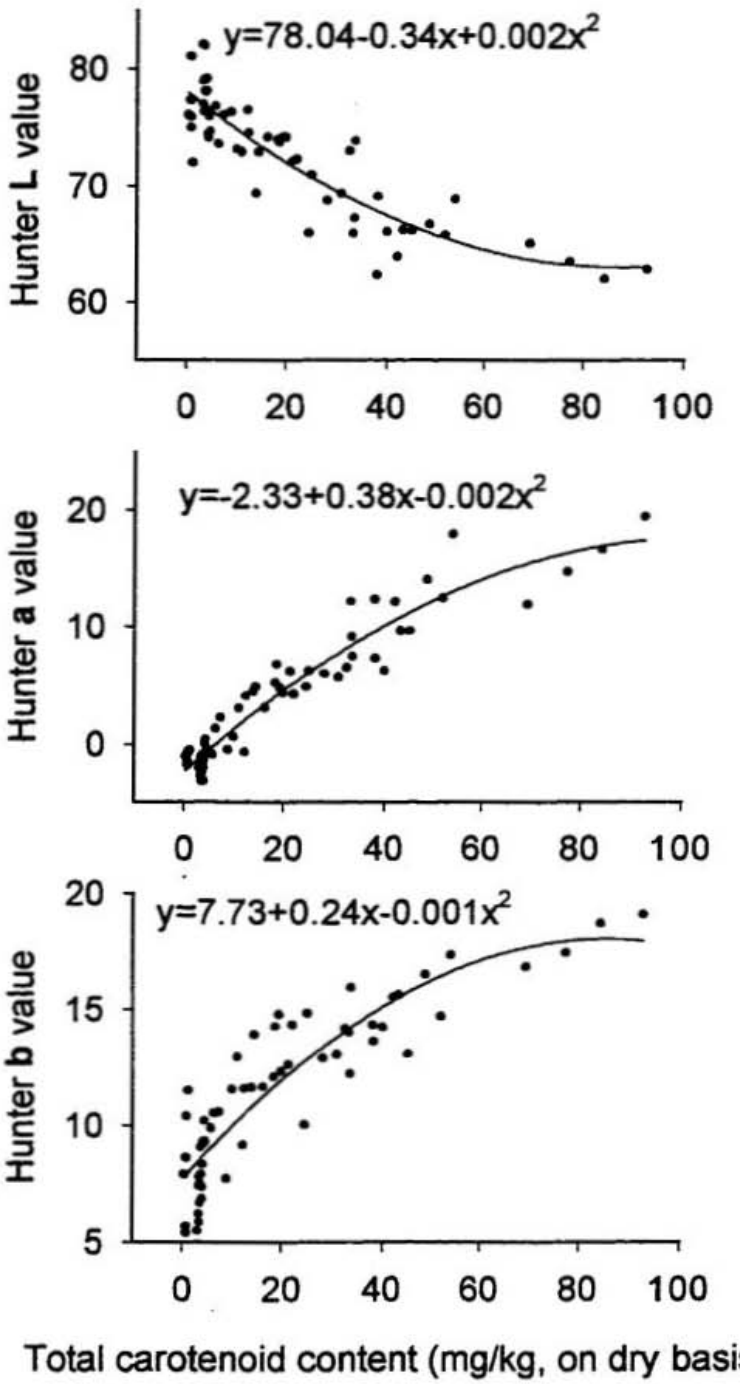

Fig. 3.19 The correlation between Hunter **L**, **a**, and **b** and total carotenoid levels in the belly skin of Arctic charr (n=60).

where x=total carotenoid contents (mg/kg, on dry basis) in flesh and belly skin, respectively.

3.3.2.3 Correlation between Hunter colour values and lipid content of the flesh

The regression analysis did not show any significant (P>0.05) relationship between any of the Hunter colour values and the lipid content of the flesh.

3.3.3 Carotenoid retention and digestibility

3.3.3.1 Retention of carotenoid in the flesh

The retention of carotenoids in the flesh of Arctic charr after feeding on experimental diets for 24 weeks is shown in Table 3.9. It is evident that carotenoid retention in the flesh decreased significantly(P<0.05) with increased levels of dietary carotenoids, while this increased (P<0.05) as dietary lipid levels increased.

Regression analysis indicated that the total amount of carotenoids retained in fish flesh was directly correlated with the amount of dietary lipid (P<0.0001), but was inversely related to the concentration of dietary carotenoids (P=0.0004), as given in the following equation:

$$y=4.60+0.29x_1-0.05x_2,\ r=0.99$$

where y=retention of carotenoid in flesh (μg retained/g growth), x_1=dietary lipid

Table 3.9 Retention of carotenoid (CR) in the flesh of Arctic charr after feeding on experimental diets for 24 weeks[1]

Diet (% lipids - ppm carotenoid)	**C R (%)**
10 - 40	5.56 ± 0.10^{d}
10 - 60	4.84 ± 0.07^{c}
10 - 80	4.13 ± 0.29^{b}
18 - 40	7.67 ± 0.23^{g}
18 - 60	6.83 ± 0.19^{f}
18 - 80	6.02 ± 0.07^{e}
26 - 40	10.66 ± 0.15^{a}
26 - 60	9.72 ± 0.06^{i}
26 - 80	8.16 ± 0.14^{h}
Dietary lipid	P<0.01
Dietary carotenoid	P<0.01
Interaction of dietary lipid and dietary carotenoid	P<0.01

[1]Results are mean values of triplicate determinations ± standard deviation. Data with different superscript are significantly different (P<0.05) from one another.

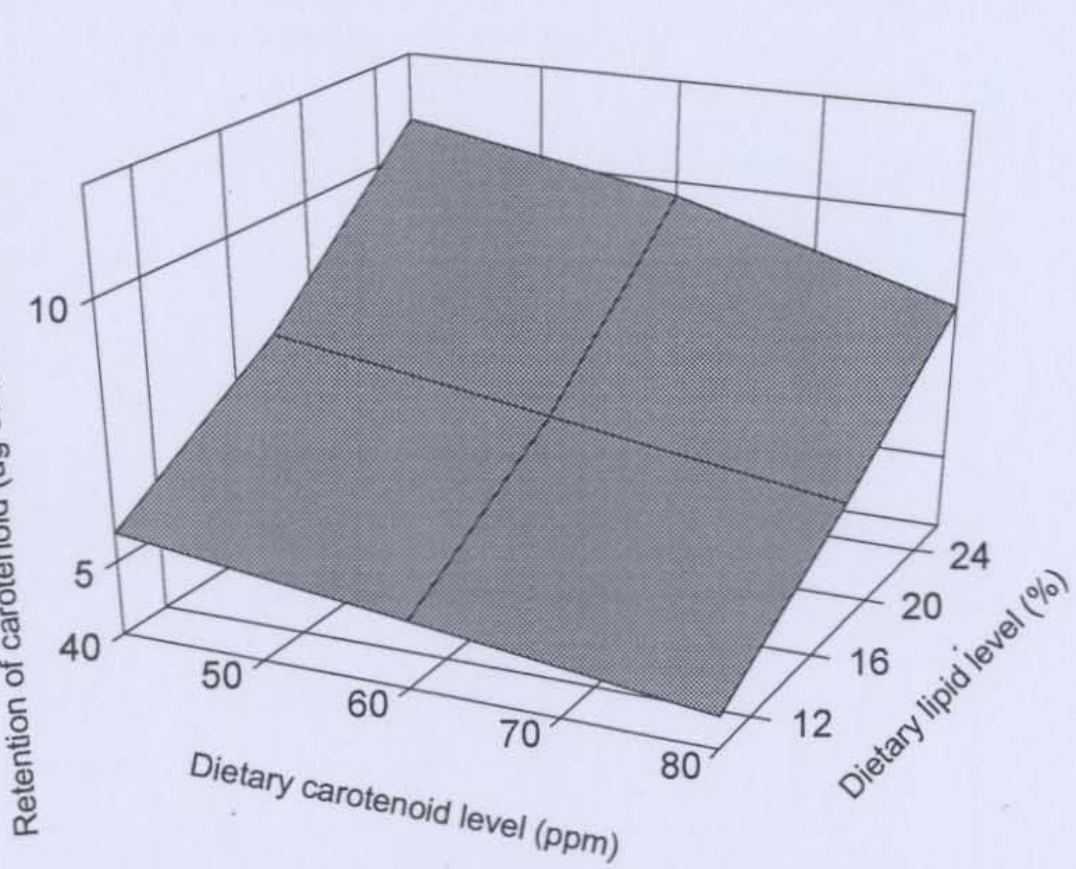

Fig. 3.20 The effects of dietary lipid (%) and carotenoid (ppm) on the retention of carotenoid in the flesh of Arctic charr.

$y=4.60+0.29x_1-0.05x_2$, where y=retention of carotenoid,

x_1=dietary lipid level, x_2=dietary carotenoid level.

Table 3.10 Apparent digestibility coefficients (ADC) of dietary carotenoid of Arctic charr fed on different diets[1]

Diet (% lipids - ppm carotenoid)	**ADC** (%)
10 - 40	60.33 ± 3.38^{d}
10 - 60	52.67 ± 2.47^{b}
10 - 80	47.99 ± 4.10^{b}
18 - 40	69.55 ± 1.47^{ac}
18 - 60	67.01 ± 3.16^{ac}
18 - 80	65.33 ± 1.33^{c}
26 - 40	73.62 ± 3.54^{a}
26 - 60	69.63 ± 2.04^{ac}
26 - 80	67.10 ± 2.25^{ac}
Dietary lipid	$P<0.01$
Dietary carotenoid	$P<0.01$
Interaction of dietary lipid and carotenoid	$P>0.05$

[1]Results are mean values of triplicate determinations ± standard deviation. Data with different superscript are significantly different ($P<0.05$) from one another.

levels, x_2=dietary carotenoid levels (Fig. 3.20).

3.3.3.2 Apparent digestibility coefficient of dietary carotenoid

The mean apparent digestibility of dietary carotenoids of Arctic charr fed on various experimental diets over a 24-week period is presented in Table 3.10. It appears that the digestibility of carotenoids decreased with a decrease in the amount of dietary lipid or an increase in the level of dietary carotenoids.

The result of regression analysis of digestibility on the level of dietary lipid and pigment indicated that digestibility of carotenoids correlated directly with the amount of dietary lipid (P=0.0014), but was inversely related with dietary pigment levels (P=0.0399), as given by the equation:

$$y=56.7+1.03x_1-0.192x_2,\ r=0.93$$

where y=apparent digestibility of carotenoid (%), x_1=dietary lipid levels, x_2=dietary pigment levels (Fig. 3.21).

3.3.3.3 Correlation between retention of carotenoid and apparent digestibility coefficient

Regression analysis revealed a relationship (P=0.0113) between the amount of carotenoids retained in the flesh and digestibility of carotenoids. The relation is

$$y=37.3-1.29x+0.013x^2,\ r=0.95$$

where y=retention of carotenoids in the flesh, x=apparent digestibility coefficient of carotenoids (Fig. 3.22).

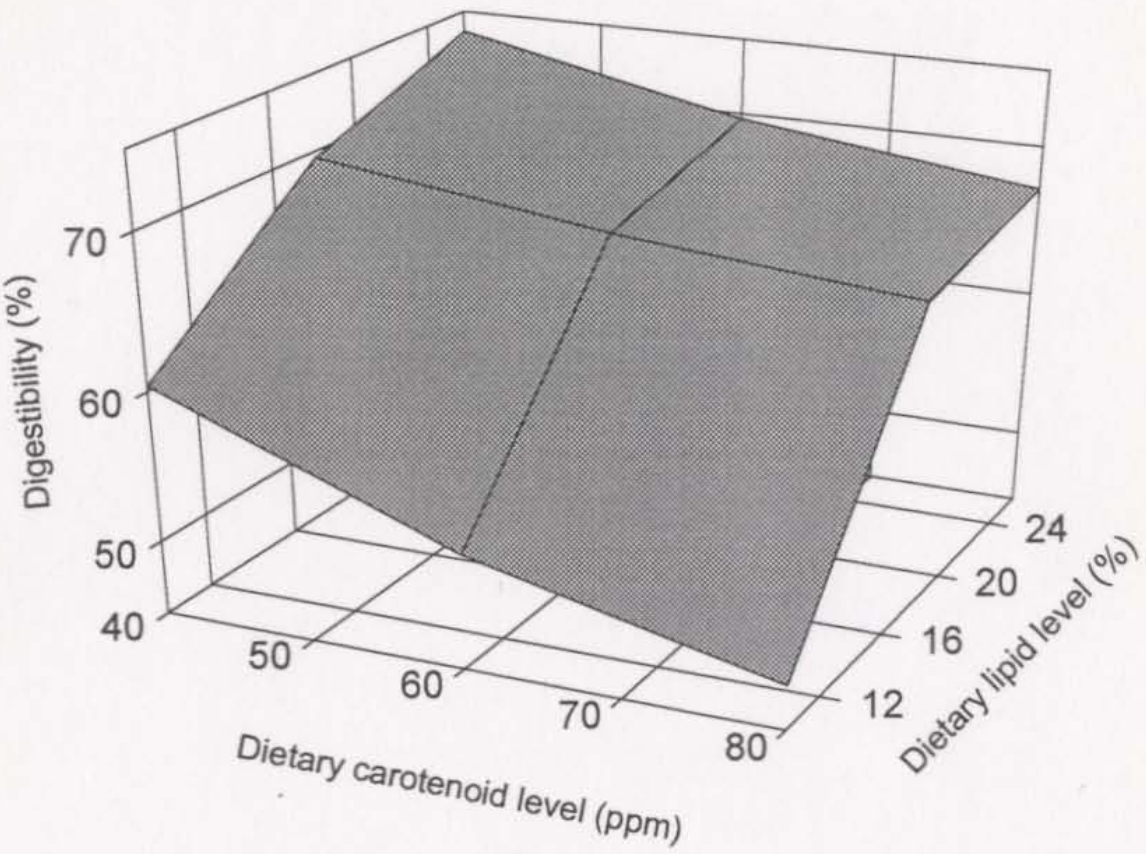

Fig. 3.21 Digestibility of Arctic charr fed experimental diets containing different lipid (%) and carotenoid (ppm) levels.
$y=56.7+1.03x_1-0.192x_2$, where y=apprant digestibility, x_1=dietary lipid level, x_2=dietary carotenoid level.

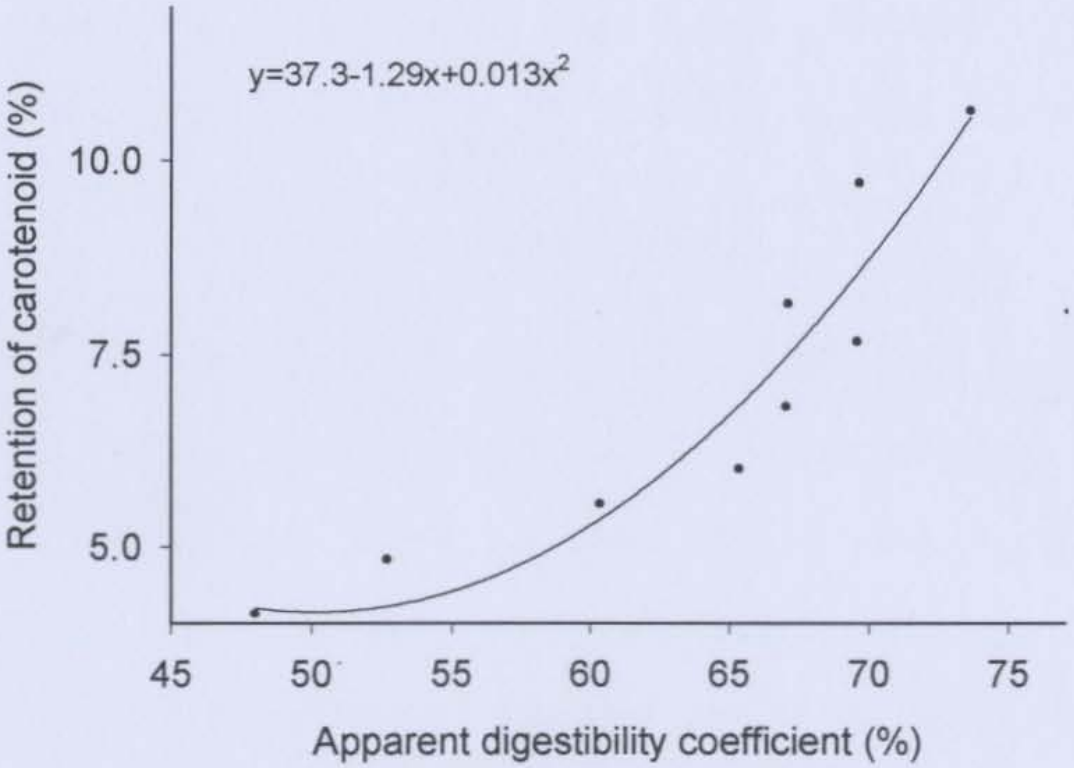

Fig. 3.22 The relation between carotenoid retention (%) in the flesh and apparent digestibility of carotenoid (%) of Arctic charr

Chapter 4

DISCUSSION

4.1 Growth

The mean specific growth rate (**SGR**) of Arctic charr in this study ranged from 0.88-1.01 % per day over a 24-week of feeding experiments. This is in agreement with previous observations under similar conditions for charr (Christiansen and Wallace, 1988). Thus, various levels of dietary lipid and carotenoids did not result in abnormal growth of fish in the present study. Increasing the proportion of dietary lipids improved the weight gain, and this was significantly greater for diets containing 18 and 26 % lipids than those containing 10 % lipid. This is in agreement with the result of Tabachek (1986) who increased the level of dietary lipids from 10 to 15 %. It is known that dietary lipids have a sparing effect on proteins (Watanabe, 1977; Takeuchi et al., 1978), thus allowing utilization of proteins for growth rather than for energy. Therefore, although the groups receiving 10 % dietary lipid were fed the same level of dietary protein as the 18 and 26 % groups, the dietary lipid level they received may not have allowed optimization of the available protein. This may be the main reason for the lower growth rate of groups fed on 10 % dietary lipid. However, too much dietary lipid may result in an imbalance of the **DE/DP** (i.e. dietary energy/dietary protein) ratio and excessive fat deposition in the visceral cavity and tissues, which would adversely affect of growth, product quality, and storage (National Research Council, 1993). It seems this did not occur in the present study,

indicating that the upper lipid limit was not reached. This corresponds with the finding of Watanabe (1982) who reported that addition of dietary lipids ranging from 5 to 25 % did not lead to any ill effects in rainbow trout or carp.

Although there was no statistically significant difference in the **SGR** of groups receiving dietary carotenoids and those on diets devoid of any dietary carotenoids, there was a tendency that at the same dietary lipid level of 18 %, the weight gains of fish in the groups fed dietary carotenoids were higher than that of fish receiving no dietary carotenoids. This growth-enhancing effect of dietary carotenoids was also found by Deufel (1965), Torrissen(1984), No and Storebakken (1991) , and Nakano et. al. (1995) for rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). This growth-enhancing effect of carotenoids may be due to a more favourable effect of astaxanthin or the carrier medium on the acceptability of the feed, resulting in a higher feed intake, or eventual physiological effects of dietary carotenoids (Bauernfeind, 1976; Tacon, 1981; Choubert, 1986; Torrisen et al., 1989; No and Storebakken, 1991).

4.2 Body composition

It was found that changes in the body composition of fish were primarily in their content of moisture and lipid (Groves, 1970). In this study, a large decrease in

moisture content and a corresponding increase in flesh lipid content and weight gain was observed, similar to that reported by Tidwel and Robinette (1990) for channel catfish (*Ictalurus punctatus*). This is likely because additional energy stored as fat simply replaces body water (Reinitz, 1983). The increased fat content in the flesh with increasing dietary lipid level coincides with the work of Ogino et al. (1976) who found that body lipid deposition is directly related to dietary lipid levels in rainbow trout. Similar results have been reported for Arctic charr (Tabachek, 1986), rainbow trout (Castledine and Buckley, 1980), plaice (Cowey et al., 1975), channel catfish (Garling and Wilson, 1976), carp (Takeuchi and Watanabe, 1979), and turbot (Bromley, 1980). At the end of the feeding experiment, the increase in moisture and decrease in lipid content of the flesh may have been due to the initiation of gonad maturation. Generally, in fish, most of the stored lipids in the somatic tissues are mobilized to gonad during sexual maturation (Dygert, 1990), thus resulting in a decrease in fat content of muscle. The decreased lipid in the muscle is normally associated with an elevated moisture content (Love, 1988).

The fatty acid composition of the dietary lipid has a significant influence on the tissue fatty acid composition of fish (Watanabe, 1982; Henderson and Tocher, 1987; Sargent et al., 1989). This may, however, apply mostly to natural and commercial diets high in total lipids which inhibit the *de novo* synthesis of fatty acids

(Sargent et al., 1989). Fish fed on low-lipid diets may modify the digested fatty acids extensively. In this study, the flesh lipid fatty acid composition of fish fed on 18 and 26 % dietary lipids basically reflected the fatty acid profile of their diets (Tables 2.4 and 3.3). Although there were differences in the content of some individual fatty acids (mainly 18:1ω9) between the flesh of fish and their diets, no significant difference in the total saturated, monounsaturated (**MUFA**) and polyunsaturated (**PUFA**) fatty acids contents were found between the content of flesh and dietary lipids. However, the total amounts of **MUFA** and **PUFA** of flesh lipid of fish fed on 10 % dietary lipid were significantly different from those of their diets, with a higher total **MUFA** and lower total **PUFA** observed for flesh lipids. The two-fold difference in 18:1ω9, which is the major end product of the *de novo* synthesis of fatty acids, between the flesh and diet with a higher content in flesh lipid indicates that some modification of digested fatty acids may have taken place (Olsen et al., 1991). It is interesting to note that the saturated fatty acids in the flesh of fish fed on various dietary lipid groups remained relatively constant at 22 to 25 % of the total, regardless of their content in the diets. Similar results were reported by Stickney and Andrews (1971). In their feeding experiment with catfish, a diet containing 10 % tallow (41% saturates) was fed to a group of catfish, and another diet containing 10 % menhaden oil (25% saturates) to a second group. After 10 weeks feeding at 20 °C water, the saturated fatty acid content

of the two groups of fish was found to be the same at about 20 % of the body lipids. Yu et al. (1977) have also indicated that the saturated fatty acids in fish body lipid remained fairly constant at about 24% of the total regardless of the high dietary saturated fatty acids. Thus, a mechanism may exist in fish to regulate and maintain a proper level of body lipid saturation (Watanabe, 1982). It is reasonable to consider that the ratio of saturated to unsaturated fatty acids largely reflects the balance of the enzyme systems producing these fatty acids (i.e. fatty acid synthetases and various desaturases and chain elongases) (Farkas et al., 1980).

The result that liver lipid content decreased when dietary lipid was increased from 18 to 26 % is in line with the observation of Tabachek (1986) for Arctic charr, but is contrary to the report of Lee and Putnam (1973) who found that, in rainbow trout, liver lipid increased as dietary lipid increased from 8 to 24 % at protein levels of 34-54%. In this study, the increase in flesh rather than liver lipid with increasing dietary lipid indicates that, when Arctic charr was fed on high levels of dietary lipid, the excess lipid appeared to be deposited within the body tissues rather than in the liver. The present results lend further support to those of Watanabe (1982) who reported that the lipid content of liver is not much affected by dietary lipid levels. The lower liver lipid content in 26 % group compared to those in 10 and 18 % group may be due to a more optimal ratio of dietary protein to lipid in the 26 % group than those

in the 10 and 18 % groups. This is because when the levels of both dietary protein and lipid approach an optimal value, both liver and whole body lipid are low (Tabachek, 1986). A significant effect ($P<0.0001$) of dietary carotenoids on the content of liver lipids was noted in this study. It is surprising to find that the liver lipid content in fish receiving no dietary carotenoid supplement was significantly ($P<0.001$) higher than those of fish fed on diets supplemented by carotenoids (Table 3.4). Although there was no statistically significant ($P>0.05$) difference in hepatosomatic indices (**HSI**) of fish fed on diets with or without dietary carotenoids, it is clear that the **HSI** of fish receiving no dietary carotenoids was higher than that of fish fed on dietary carotenoids (Table 3.4). Unfortunately, no prior literature reference is available concerning the effect of dietary carotenoids on liver lipids of fish for comparative purposes. However, the observation of Nakano et al. (1995) may indirectly lend support to the present observations. These authors observed the biochemical characteristics of liver and blood of rainbow trout (*Oncorhynchus mykiss*), fed on a diet supplemented with red yeast (*Phaffia rhodozyma*) which contains astaxanthin as its principal carotenoid pigment or synthetic astaxanthin (the pigment also used in present study). It was found that the hepatosomatic indices and the activities of serum glutamic-oxaloacetic transaminase of fish fed on a diet containing red yeast or synthetic astaxanthin was significantly lower than those of fish fed on a control diet

(Nakano et al., 1995). The mean amount of serum lipid peroxide of fish fed on a diet containing red yeast or synthetic astaxanthin was also lower than that of the control fish. These results suggest that dietary carotenoids have the potential of improving, not only the pigmentation of fish muscle but also, the health of fish in aquaculture (e.g. improvement of liver function and enhancement of defensive potential against oxidative stress).

4.3 Pigmentation

Even though there are some indications of a biological function of carotenoids in fishes (Tacon, 1981; Torrissen, 1984), the main purpose of feeding carotenoid to salmonids is to achieve an acceptable pigmentation in the product for marketing. It has been reported that the flesh of farmed salmonids should have at least 3 - 4 mg/kg of total carotenoids in order to be marketable (Torrissen et al., 1989; Shahidi et al., 1993). Based on this criterion, after 20 weeks of feeding, the final carotenoid concentration in the flesh of all fish receiving carotenoids ranged from 3.90 to 9.38 mg/kg of wet tissue, which is sufficient to confer to the product a natural, pink colour for marketing.

Muscle pigmentation depends on the species and size of fish. In sockeye salmon, for example, pigmentation is evident in fish weighing only 20 g

(Yarxhombek, 1970). In coho salmon, Spinelli and Mahnken (1978) demonstrated that adequate pigmentation occurred for fish weighing about 200 g. Arai et al. (1987) confirmed the results of Spinelli and Mahnken (1978) that coho salmon weighing about 80 g were virtually unable to assimilate dietary astaxanthin in their flesh when raised in fresh water. In contrast, fish weighting around 180 g were effectively pigmented when fed on diets containing krill oil. However, rainbow trout, *Salmo gairdneri*, weighting 40 - 80 g failed to deposit carotenoids in their musculature after being fed on a diet containing capelin oil with a 50 mg/kg carotenoid content (Chouber and Luquet, 1975). The results of Torrissen (1985) confirmed the above observation and demonstrated the inability of rainbow trout, *S. gairdneri*, weighting 100 - 150 g to deposit carotenoids in their flesh. However, Christiansen and Wallace (1988) found that Arctic charr, at a body weight of 17 g, deposited canthaxanthin in their muscle after being fed on a diet containing 40 mg/kg dietary canthaxanthin for 63 days. The results of this work also demonstrated that fish weighting 65 - 75 g is large enough to show muscle pigmentation. Therefore, the minimum fish size required for muscle pigmentation may be lower in Arctic charr.

Torrissen et al. (1989) discussed two hypothetical models for pigment deposition in salmonoids. In the first, flesh carotenoids concentration and their retention efficiency are considered to be a linear function of fish weight. Based on the

model, pigmentation of farm-raised salmonids should start relatively late in the production cycle, with the shorter pigmentation time compensated for by a higher level of carotenoids in the diet. In the second hypothetical model, it is assumed that pigment retention is constant throughout the life cycle of fish. This model predicts a rapid increase in pigment level in small fish and a plateau in pigment concentration as the fish get larger. Using this model, pigmentation should be started early in the production cycle, with the level of pigment in the feed determining the final pigment level in the fish. In this study, the result of regression analysis for the relation between fish weight and flesh pigmentation (Fig. 3.7) showed that the deposition of carotenoids in the flesh of Arctic charr fit the curvilinear retention model (i.e. the second model as mentioned above) regardless of dietary carotenoid level used in the trial. This is comparable with that for rainbow trout. A study by Storebakken et al. (1986) strongly suggests that for rainbow trout, above a certain size (0.5 - 1 kg), pigment retention is fairly constant. In comparison, coho salmon followed a linear model for the deposition of astaxanthin in their flesh (Smith et al., 1992).

Among several factors which influence carotenoid absorption, retention, and metabolism in salmonids, dietary carotenoid levels are the major determinants that control the intensity of flesh pigmentation (Torrissen et al., 1989). The result of this study, as expected, demonstrated that the diets rich in carotenoids as compared with

those in low levels of carotenoids gave a higher pigment level in the flesh with progression of time regardless of the level of dietary lipids (Fig. 3.3). The level of carotenoids in the flesh of fish on various treatments generally exceeded 4 mg/kg, after 12 - 20 weeks, which may be regarded as sufficient for visual colour impression in farmed salmon (Torrissen et al., 1989). The pigmentation time in the present study was longer than that reported by Shahidi et al. (1993). In their experiment, the level of carotenoid in the flesh of Arctic charr fed on 65 mg/kg astaxanthin exceeded 4 mg/kg after 9 weeks of pigmentation. This may be due to the different fish size used. The fish used in that study were about 10-times larger (size at start: 500-700 g) than those in the present study (starting with 65-75 g). It was reported that the apparent digestibilities of dietary carotenoids were higher for large charr than those for small charr (Christiansen and Wallace, 1988). The conclusion that the total carotenoid level in the flesh of fish from all treatments either remained relatively constant or declined (Fig. 3.3; Appendix 4) after 20 weeks of pigmentation indicates that the pigmentation reaches a plateau level, at about 4 to 9 mg/kg of wet flesh of charr fed different levels of dietary carotenoids and lipids. Similar results were obtained when rainbow trout (Chouber and Luquet, 1982; Choubert, 1983; Storebakken et al., 1986) and Atlantic salmon (Torrissen and Torrissen , 1984; Torrissen et al., 1984) were used. However, the saturation level might be influenced by genetic factors (Torrissen and Naevdal,

1984), by dietary level (Torrissen, 1985), fish size and species. In relation to other species, it is interesting to note that Torrissen et al. (1984) showed a flesh pigment level for family groups of Atlantic salmon of 4 to 5 mg/kg after 26 months of feeding, whereas Torrissen and Naevdal (1984) showed that families of rainbow trout attained a level of 5.5 to 6.5 mg/kg pigment after about 5 to 6 months of feeding on a canthaxanthin-containing diet. The present results indicate that ability of Arctic charr to accumulate carotenoids is similar to that of rainbow trout, and better than that of Atlantic salmon. It also demonstrates that the saturation level of carotenoids in the flesh might be affected, not only by dietary carotenoid level (Torrissen, 1985), but also by dietary lipid level. The declining pigmentation in the groups fed on high dietary lipid cannot be explained simply by carotenoid saturation in the tissues, as it occurred in a similar manner for all three dietary carotenoid levels. The influence of sexual maturation may have also contributed to this pigmentation decline. It is reported that in female salmonids, the carotenoids are transferred from the flesh to the gonads, whereas in the males they are conveyed to the skin (Crozier, 1970).

Contrary to the total carotenoid level in flesh, carotenoid retention (the proportion of consumed carotenoid which is deposited) in the flesh showed a tendency to decrease with increasing dietary carotenoid concentration. This agrees with previous findings of Torrissen (1985) and Choubert and Storebakken (1989), and

supports the assumption that the carotenoid retention in salmonids is inversely related to the dietary carotenoid concentration (Spinelli and Mahnken, 1978; Kotic et al., 1979; Torrisen et al., 1981; Choubert, 1983; Torrissen, 1985; Storebakken et al., 1987; Choubert and Storebakken, 1989; Torrissen et al., 1990). The reason for this decline may be due to limitations in absorption of carotenoids from the intestines (De la Noue et al., 1980). It was found that carotenoids in fish are absorbed by a specific process (Hardy et al., 1990). The observation in this study that the apparent digestibility of carotenoids decreased with increasing dietary carotenoid levels further confirms the limited absorption of dietary carotenoids. Except for the limitation in absorption, other factors such as the limitation in their transport by serum lipoproteins (Nakamura et al., 1985; Torrissen, 1986), catabolism (Foss et al., 1984; Choubert, 1985), direction into different tissues (Sivtseva and Dubrovin, 1980) or limited binding capacity to the muscle (carotenoids are bound to actomyosin)(Henmi et al., 1987,1989) might also result in decreasing retention of carotenoids.

Since carotenoids are lipid-soluble pigments, an increase in lipid content of the diet should allow a better fixation of pigments by fish. The present results showed, as expected, that the deposition of carotenoids in the flesh increased significantly ($P<0.05$) with increasing levels of lipid in the diets. This result coincides with the observations of Abdul-Malak et al. (1975), Spinelli (1979), Seurman et al. (1979) and

Torrissen (1985), but is contrary to that obtained by Choubert and Luquet (1983). This may be due to the relatively narrow range of lipid amounts in the diets (9.4 - 17.4 %) used in Choubert and Luquet's (1983) study, whereas in the present experiment, the dietary lipid content ranged from 10 to 26 %. A positive correlation ($r = 0.92$) between carotenoid concentration and lipid content of flesh was found in the present study. This is contrary to the findings of Kanemitsu and Aoe (1958), Saito (1969), Spinelli and Mahnken (1978), Foss et al. (1987), McCallum et al. (1987), and No and Storebakken (1991) for Atlantic salmon, coho salmon, rainbow trout and sea trout, respectively, but agrees, in part, with the observation of Christiansen and Wallace (1988) who found that in small (1+, 17.1 - 30.9 g) charr there was a positive correlation between canthaxanthin and lipid deposition in the muscle tissue. Again, this was not the case for large size (2+, 125.6 - 202.9 g) charr. In farmed rainbow trout, a positive correlation existed between canthaxanthin and lipid content in the musculature (Abdul-Malak et al., 1975). A decline in both astaxanthin and lipid (mainly triacylglycerol) in the muscle of chum salmon on the upstream migration to spawning has been noted by Ando et al. (1985). Therefore, the available literature on pigmentation of salmonids is somewhat fragmentary and contradictory. So far, there has been no clear picture of any relationship between lipid distribution and carotenoid deposition in the muscle tissue of salmonids. Despite inconsistent observations

reported previously, the presence of a direct relationship in the present study between the carotenoid concentration, dietary lipid level, and lipid content in flesh clearly demonstrates that high dietary lipid content can improve both the absorption of carotenoid from the intestines and their deposition in the flesh of charr. Christiansen and Wallace (1988) reported that carotenoid deposition corresponded with lipid deposition in small charr. The increase in apparent digestibility of carotenoids with increasing dietary lipid content (Fig. 3.21), which is similar to the results of Torrissen et al (1989), further confirms the improvement of utilization of dietary carotenoids by increased content of lipids in diets used in this study.

The apparent digestibility coefficients for carotenoids in Arctic charr are consistent with the values of 50 -70 % found for astaxanthin with rainbow trout (No and Storebakken, 1991), and with Atlantic salmon (Storebakken et al., 1987), but it is higher than the values of 17.9 - 38.7 % previously reported for canthaxanthin-containing feeds used for Arctic charr (Christiansen and Wallace, 1988) and 25 - 45% for canthaxanthin for rainbow trout (Torrissen et al., 1990). This may be due to the different fish size and type of carotenoids used. It was reported that the apparent carotenoid digestibility was higher for large size fish than that for small fish (Christiansen and Wallace, 1988), and that astaxanthin was utilized more efficiently than canthaxanthin for pigmentation of rainbow trout (No and Storebakken, 1992) and

Arctic charr (Shahidi et al., 1993).

The carotenoid retention (i.e. the proportion of consumed carotenoid which is deposited) in salmonids are widely variable. Kotik et al. (1979) reported a retention rate of carotenoids between 20 and 60 %, while the results of Storebakken et al. (1987) suggested a value of 1.7 to 5 %. The carotenoid retention obtained in this study ranged from 4.13 to 10.66 %. These values are comparable to those reported by Choubert (1977), Choubert and Luquet (1982), Foss et al. (1984), Choubert and Storebakken (1989) and Torrissen et al. (1989), but are lower than those published by Torrissen and Breakkan (1979) and Smith et al. (1992).

There is a difference of 40 - 65 % between the retention and the apparent digestibility of carotenoids in this study. The high apparent digestibility coefficients were caused by a low level of carotenoid in the faeces. This may be due to the decomposition of astaxanthin in the intestine, or that the absorbed astaxanthin was metabolized to non-carotenoid compounds. Hardy et al. (1990) demonstrated that metabolism of canthaxanthin takes place in the liver of rainbow trout and that the metabolized products are excreted in the bile. Metusalach et al. (1996) have recently shown that canthaxanthin may be reductively metabolized in different organs of charr. In addition, it is possible that incomplete extraction of carotenoids from the faeces might have been responsible for this observation. Experiments with labelled

astaxanthin have shown that complete carotenoid extraction from faeces is difficult to achieve (Foss et al., 1987). Although precautions were taken (the faeces were rapidly frozen at - 80 °C and freeze-dried in the dark as suggested by No and Storebakken (1991)) to avoid decomposition of the carotenoids, it still is possible that some of the astaxanthin was disintegrated during processing of the samples. These would automatically lead to reduced values for carotenoids in the faeces and, consequently an overestimation of apparent digestibility. It is also well known that chromium oxide, the usual indicator in digestibility studies, influences digestibility (Tacon and Rodrigues, 1984). Therefore, It is questionable that traditional digestibility studies can be used for labile molecules such as carotenoids (Christiansen and Wallace, 1988). However, the high correlation ($r = 0.95$) between digestibility and retention of carotenoids in the present study shows that this method of digestibility determination (Austreng, 1978) still provides a valuable means in assessing relative differences in availability of dietary carotenoids (Tacon and Rodrigues, 1984; Torrissen et al., 1990; No and Storebakken, 1991).

Besides the chemical analysis, colour measurement was also used to study the pigmentation of Arctic charr. The results of the present study clearly revealed a curvilinear relationship between Hunter **L**, **a** and **b** values and carotenoid concentrations in the flesh and belly skin of charr. This correspondence is similar to

that reported for coho salmon (Smith et al, 1992) and Atlantic salmon (Christiansen et al., 1995). The fact that the intensity of redness (Hunter **a** values) increased while Hunter **L** values decreased concurrently with increasing carotenoid concentrations in fish flesh is in agreement with previous findings (Schmidt and Idler, 1958; Saito, 1969; Skrede and Storebakken, 1986a,b; Smith et al., 1992). The curvilinear relationship describing Hunter **a** value and carotenoid concentration in the flesh is in contrast to the linear relationship reported by Skrede and Storebakken (1986a,b), and No and Storebakken (1991). A significant relationship also existed between the yellowness (Hunter **b** value) and carotenoid content in fish flesh and this is in agreement with the observation of No and Storebakken (1991), but in contrast to that reported by Skrede and Storebakken (1986b). These discrepancies could be attributed to the existing differences in sampling and measuring procedures and also the species of fish used. It was reported that measuring conditions strongly influence the results obtained from instrumental analysis for flesh colour evaluation of salmonids (Little and MacKinney, 1969; Schmidt and Cuthbert, 1969; Choubert, 1982; Skrede and Storebakken, 1986a).

Schmidt and Idler (1958) reported that canned sockeye salmon with a higher fat content in the flesh had a more desirable colour. In this study, however, the regression analysis did not reveal any significant relationship between any of the

Hunter colour values and lipid content in flesh. Similar findings were reported by No and Storebakken (1991). This may be due to the relatively narrow range of flesh lipid contents in the present study.

In conclusion, the results of present study has clearly demonstrated that the increase in both lipid and carotenoid levels in the diet of Arctic charr enhances the deposition of carotenoids in their flesh and skin. There was also a significant interaction of dietary lipid and carotenoids on the pigmentation of Arctic charr. An increase in dietary lipid within a certain range improves the flesh pigmentation of charr.

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Appendix 1. The growth data of Arctic charr fed on diets contianing different combinations of dietary lipids and carotenoids over 24 weeks

Time interval (weeks)		Diet (% lipid - ppm carotenoid)									
		18 - 0[3]	10 - 40	10 - 60	10 - 80	18 - 40	18 - 60	18 - 80	26 - 40	26 - 60	26 - 80
initial	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
4	Weight(g)[1]	88.78	91.22	95.41	92.73	96.28	91.45	97.15	98.88	95.93	97.86
	Length(cm)[2]	17.1± 1.2	18.0 ± 1.3	17.2 ± 1.1	17.4 ± 1.2	18.7 ± 1.4	17.9 ± 1.3	17.7 ± 1.0	17.2 ± 1.4	17.9 ± 1.6	17.5 ± 1.4
8	Weight(g)[1]	119.12	122.40	128.74	124.08	129.92	125.14	130.72	136.06	132.74	132.78
	Length(cm)[2]	18.6± 1.2	19.8 ± 1.4	19.4 ± 1.1	19.1 ± 1.0	19.4 ± 1.1	18.6 ± 1.5	18.2 ± 1.2	20.0± 1.1	18.9 ± 1.2	19.9 ± 1.6
12	Weight(g)[1]	158.50	159.70	167.04	160.54	173.83	169.33	174.91	185.13	180.12	179.16
	Length(cm)[2]	20.5± 1.3	21.5 ± 1.2	20.0 ± 1.1	20.6 ± 1.0	20.8± 1.6	20.8 ± 1.0	19.7 ± 1.7	21.2 ± 1.8	20.8 ± 1.5	20.6 ± 1.1
16	Weight(g)[1]	205.07	201.48	213.71	206.55	227.44	220.93	230.78	246.33	240.33	235.73
	Length(cm)[2]	22.4± 1.2	22.8 ± 1.3	21.7 ± 1.1	23.0 ± 1.5	23.8 ± 1.2	22.6 ± 1.3	23.1 ± 1.3	23.2 ± 1.3	22.4 ± 1.4	22.1 ± 1.1

Appendix 1. The growth data of Arctic charr fed on diets contianing different combinations of dietary lipids and carotenoids over 24 weeks (continued)

Time interval (weeks)		Diet (% lipid - ppm carotenoid)									
		18 - 0[3]	10 - 40	10 - 60	10 - 80	18 - 40	18 - 60	18 - 80	26 - 40	26 - 60	26 - 80
20	Weight(g)[1]	263.10	250.65	266.62	260.59	295.09	290.69	301.96	317.82	315.33	309.30
	Length(cm)[2]	24.7± 1.2	24.9 ± 1.4	24.7 ± 1.3	25.0 ± 1.6	25.8 ± 1.6	26.8 ± 1.3	26.2 ± 1.8	26.9 ± 1.2	27.8 ± 1.5	26.4 ± 1.4
24	Weight(g)[1]	328.24	301.53	321.64	317.01	367.12	364.69	373.56	386.63	386.84	380.51
	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 (%)[4]	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]

[1]Average body weight in each tank, the fish were weighted in bulk. [2]Results are mean values of 8-10 determination ± standard deviation. Data in last row with different superscript are significantly different ($p<0.05$) from one another. [3]Contorl. [4]Weight gain=(final weight-initial weight)÷initial weight×100.

Appendix 2. The mean specific growth rate (SGR) of Arctic charr fed diets containing different combinations of dietary lipids and carotenoids during given time intervals[1]

Diet (% lipid-ppm carotenoid)	Specific Growth Rate (G_w% per day)						
	Time interval (weeks)						
	0 - 4	5 - 8	9 - 12	13 - 16	17 - 20	21 - 24	Average (0 - 24)
10 - 40	1.01^a	1.05^a	0.95^b	0.83^b	0.78^b	0.66^b	0.88^b
10 - 60	1.05^a	1.07^a	0.93^b	0.88^b	0.79^b	0.67^b	0.90^b
10 - 80	0.98^a	1.04^a	0.92^b	0.90^b	0.83^b	0.70^b	0.90^b
18 - 40	1.03^a	1.07^b	1.04^a	0.96^a	0.93^a	0.78^a	0.97^a
18 - 60	1.09^a	1.12^b	1.08^a	0.95^a	0.98^a	0.81^a	1.01^a
18 - 80	1.08^a	1.06^b	1.04^a	0.99^a	0.96^a	0.76^a	0.98^a
26 - 40	1.20^a	1.14^a	1.10^a	1.02^a	0.91^a	0.70^b	1.01^a
26 - 60	1.15^a	1.16^a	1.09^a	1.03^a	0.97^a	0.73^b	1.02^a
26 - 80	1.03^a	1.09^a	1.07^a	0.98^a	0.97^a	0.74^b	0.98^a
Control	1.04^a	1.05^b	1.02^a	0.92^a	0.89^a	0.79^a	0.95^a
Dietary lipids	$P > 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.05$	$P < 0.01$
Dietary carotenoids	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

[1]Data in each column with different superscript are statistically different ($p<0.05$) from one another.

Appendix 3. Proximate composition (%) of the flesh of Arctic char fed with different diets[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
	Moisture content					
18 - 0	79.49 ± 0.11^{ax}	78.00 ± 0.14^{cx}	74.97 ± 0.11^{dx}	72.06 ± 0.01^{cy}	68.30 ± 0.06^{by}	68.50 ± 0.22^{by}
10 - 40	79.13 ± 0.16^{ax}	77.99 ± 0.22^{fx}	75.15 ± 0.25^{ex}	73.06 ± 0.37^{dx}	70.08 ± 0.13^{bx}	70.78 ± 0.07^{cx}
10 - 60	78.96 ± 0.24^{ax}	78.51 ± 0.18^{fx}	73.90 ± 0.79^{ex}	72.34 ± 0.05^{dx}	70.54 ± 0.32^{cx}	70.06 ± 0.02^{bx}
10 - 80	79.23 ± 0.00^{ax}	76.99 ± 0.17^{dx}	74.90 ± 0.01^{cx}	72.30 ± 0.07^{bx}	69.94 ± 0.12^{bx}	70.00 ± 0.18^{bx}
18 - 40	78.80 ± 0.21^{ax}	78.11 ± 0.04^{fx}	73.70 ± 0.42^{ex}	71.87 ± 0.09^{dy}	69.38 ± 0.14^{cy}	68.77 ± 0.33^{by}
18 - 60	79.36 ± 0.36^{ax}	78.08 ± 0.11^{fx}	73.74 ± 0.11^{ex}	71.08 ± 0.24^{dy}	68.26 ± 0.51^{by}	69.93 ± 0.66^{cy}
18 - 80	78.67 ± 0.07^{ax}	78.56 ± 0.04^{ax}	73.64 ± 0.83^{ax}	71.61 ± 0.00^{by}	68.67 ± 0.24^{by}	68.71 ± 0.72^{by}
26 - 40	79.14 ± 0.15^{ax}	76.49 ± 0.01^{fy}	73.14 ± 0.08^{ey}	69.47 ± 0.15^{dz}	66.25 ± 0.42^{bz}	67.82 ± 0.30^{cz}
26 - 60	78.33 ± 0.09^{ax}	75.97 ± 0.28^{fy}	71.26 ± 0.02^{ey}	69.17 ± 0.06^{dz}	65.37 ± 0.39^{bz}	67.11 ± 0.81^{cz}
26 - 80	79.42 ± 0.06^{ax}	76.52 ± 0.09^{fy}	71.23 ± 0.26^{ey}	68.55 ± 0.33^{dz}	66.44 ± 0.53^{bz}	67.77 ± 0.11^{cz}
Dietary lipid	P>0.05	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01
Dietary carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Interaction of dietary lipid and carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

[1]Results are mean values of triplicates determination ± standard deviation. Values in each column with different superscript (a-f) and in each row with different superscript (x,y,z) are significantly different (P<0.05) from one another, respectively. The flesh of char prior to experiment contained 79.89 ± 0.15% of moisture.

Appendix 3. Proximate composition (%) of the flesh of Arctic char fed with different diets (continuted I)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
	Protein content					
18 - 0	16.71 ± 0.25^{bx}	17.36 ± 0.21^{cx}	18.95 ± 0.17^{dx}	18.70 ± 0.30^{dx}	18.17 ± 0.61^{dx}	19.89 ± 0.43^{axy}
10 - 40	17.83 ± 0.03^{bx}	17.81 ± 0.03^{bx}	19.22 ± 0.00^{cx}	21.46 ± 0.19^{ax}	20.07 ± 0.88^{cx}	20.87 ± 0.13^{cx}
10 - 60	18.15 ± 0.14^{ax}	18.14 ± 0.53^{ax}	19.93 ± 0.11^{ax}	20.30 ± 0.94^{ax}	20.59 ± 0.80^{ax}	20.57 ± 0.51^{ax}
10 - 80	17.92 ± 0.05^{bx}	18.71 ± 0.10^{cx}	20.09 ± 0.05^{cdx}	20.62 ± 0.15^{adx}	19.78 ± 0.68^{dcx}	22.03 ± 1.26^{ax}
18 - 40	18.03 ± 0.07^{bx}	17.67 ± 0.07^{bx}	19.25 ± 0.35^{bx}	19.40 ± 0.49^{bx}	17.90 ± 1.63^{bx}	20.62 ± 0.48^{axy}
18 - 60	17.42 ± 0.29^{ax}	16.22 ± 1.97^{ax}	19.29 ± 0.68^{ax}	20.17 ± 1.59^{ax}	19.61 ± 1.91^{ax}	20.64 ± 0.38^{axy}
18 - 80	17.83 ± 0.15^{ax}	16.65 ± 0.98^{bx}	19.57 ± 0.37^{ax}	19.35 ± 0.43^{ax}	19.87 ± 0.43^{ax}	19.53 ± 0.49^{axy}
26 - 40	16.81 ± 0.88^{bx}	17.82 ± 0.30^{cx}	19.44 ± 0.51^{ax}	18.76 ± 0.71^{acx}	19.74 ± 0.15^{ax}	18.42 ± 0.43^{acy}
26 - 60	18.12 ± 0.44^{ax}	17.62 ± 0.12^{ax}	20.04 ± 0.03^{ax}	19.97 ± 1.21^{ax}	19.14 ± 1.07^{ax}	18.97 ± 2.73^{ay}
26 - 80	17.07 ± 0.49^{ax}	18.31 ± 0.10^{ax}	19.98 ± 0.64^{ax}	19.93 ± 0.12^{ax}	19.50 ± 0.36^{ax}	19.10 ± 0.91^{ay}
Dietary lipid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P<0.05
Dietary carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Interaction of dietary lipid and carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

[1]Results are mean values of triplicates determination ± standard deviation. Values in each column with different superscript (a-f) and in each row with different superscript (x,y,z) are significantly different (P<0.05) from one another, respectively. The flesh of char prior to experiment contained 16.01 ± 1.48% of protein.

Appendix 3. Proximate composition (%) of the flesh of Arctic char fed with different diets (continuted II)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
	Lipid content					
18 - 0	1.84 ± 0.29^{bx}	3.09 ± 0.44^{cx}	4.34 ± 0.35^{dy}	5.93 ± 0.33^{ey}	9.87 ± 0.25^{ay}	8.67 ± 0.37^{fy}
10 - 40	1.92 ± 0.13^{bx}	2.76 ± 0.53^{cx}	3.71 ± 0.13^{dz}	4.37 ± 0.26^{ez}	8.26 ± 0.17^{az}	5.72 ± 0.23^{fz}
10 - 60	1.63 ± 0.24^{bx}	1.97 ± 0.17^{bx}	4.18 ± 0.34^{cz}	5.74 ± 0.41^{dz}	7.19 ± 0.09^{az}	6.39 ± 0.14^{ez}
10 - 80	1.63 ± 0.33^{bx}	2.18 ± 0.28^{bx}	3.34 ± 0.21^{cz}	5.28 ± 0.35^{dz}	8.21 ± 0.12^{az}	6.35 ± 0.20^{ez}
18 - 40	2.00 ± 0.11^{bx}	2.30 ± 0.11^{cx}	4.26 ± 0.43^{dy}	6.28 ± 0.18^{ey}	10.00 ± 0.21^{ay}	8.11 ± 0.29^{fy}
18 - 60	1.77 ± 0.37^{bx}	3.54 ± 0.19^{cx}	5.16 ± 0.53^{dy}	7.13 ± 0.36^{ey}	10.54 ± 0.31^{ay}	7.94 ± 0.16^{fy}
18 - 80	2.19 ± 0.09^{bx}	2.47 ± 0.23^{bx}	4.98 ± 0.29^{cy}	6.93 ± 0.24^{dy}	10.05 ± 0.09^{ay}	8.48 ± 0.38^{ey}
26 - 40	2.03 ± 0.16^{bx}	3.01 ± 0.09^{cx}	6.10 ± 0.17^{dx}	9.27 ± 0.28^{ex}	12.28 ± 0.20^{ax}	10.84 ± 0.41^{fx}
26 - 60	2.15 ± 0.07^{bx}	4.22 ± 0.88^{cx}	7.36 ± 0.58^{dx}	9.10 ± 0.15^{ex}	12.54 ± 0.46^{ax}	10.48 ± 0.26^{fx}
26 - 80	2.09 ± 0.11^{bx}	3.44 ± 0.24^{cx}	6.87 ± 0.29^{dx}	9.69 ± 0.36^{ex}	11.12 ± 0.13^{ax}	9.98 ± 0.15^{ex}
Dietary lipid	P>0.05	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01
Dietary carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Interaction of dietary lipid and carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

[1]Results are mean values of triplicates determination ± standard deviation. Values in each column with different superscript (a-f) and in each row with different superscript (x,y,z) are significantly different (P<0.05) from one another, respectively. The flesh of char prior to experiment contained 1.58 ± 0.31% of lipids.

Appendix 3. Proximate composition (%) of the flesh of Arctic char fed with different diets (continuted III)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
			Ash content			
18 - 0	1.11 ± 0.03^{a}	1.19 ± 0.01^{a}	1.14 ± 0.02^{a}	1.18 ± 0.02^{a}	1.20 ± 0.03^{a}	1.16 ± 0.0^{a}
10 - 40	1.16 ± 0.01^{a}	1.13 ± 0.06^{a}	1.26 ± 0.01^{a}	0.92 ± 0.06^{b}	1.31 ± 0.02^{a}	1.15 ± 0.03^{a}
10 - 60	0.97 ± 0.02^{d}	0.74 ± 0.02^{c}	1.09 ± 0.01^{a}	1.06 ± 0.01^{f}	1.00 ± 0.01^{d}	0.68 ± 0.03^{b}
10 - 80	1.13 ± 0.02^{b}	1.33 ± 0.04^{a}	1.21 ± 0.04^{ab}	1.19 ± 0.01^{ab}	1.17 ± 0.01^{b}	1.13 ± 0.02^{b}
18 - 40	1.07 ± 0.02^{c}	0.83 ± 0.05^{b}	1.05 ± 0.01^{c}	0.94 ± 0.03^{c}	1.19 ± 0.03^{a}	1.08 ± 0.03^{c}
18 - 60	1.16 ± 0.02^{c}	1.06 ± 0.02^{cd}	1.34 ± 0.03^{a}	1.25 ± 0.02^{f}	1.00 ± 0.05^{bc}	0.94 ± 0.05^{b}
18 - 80	0.95 ± 0.01^{c}	0.64 ± 0.04^{b}	1.01 ± 0.02^{c}	1.12 ± 0.01^{ac}	1.18 ± 0.01^{a}	1.12 ± 0.02^{ac}
26 - 40	1.11 ± 0.03^{d}	0.91 ± 0.01^{b}	1.13 ± 0.05^{d}	1.24 ± 0.04^{a}	1.16 ± 0.02^{d}	1.05 ± 0.01^{c}
26 - 60	1.09 ± 0.05^{ab}	1.02 ± 0.01^{b}	1.09 ± 0.01^{ab}	1.18 ± 0.01^{a}	1.12 ± 0.08^{ab}	1.04 ± 0.05^{b}
26 - 80	1.12 ± 0.02^{bc}	1.22 ± 0.03^{b}	1.33 ± 0.04^{b}	1.43 ± 0.03^{a}	1.20 ± 0.04^{b}	1.11 ± 0.05^{bc}
Dietary lipid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Dietary carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Interaction of dietary lipid and carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

[1]Results are mean values of triplicates determination ± standard deviation. Values in each column with different superscript (a-f) are significantly different ($P<0.05$) from one another. The flesh of char prior to experiment contained 1.13 ± 0.01% of ash.

Appendix 4A. Total carotenoid content (mg/kg, on wet basis) in the flesh of Arctic char after given time pigmentation[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
18 - 0	0.17 ± 0.08^{aq}	0.22 ± 0.16^{aq}	0.41 ± 0.18^{aq}	0.52 ± 0.25^{aq}	0.27 ± 0.09^{aq}	0.30 ± 0.13^{aq}
10 - 40	0.24 ± 0.10^{br}	0.35 ± 0.08^{bs}	1.45 ± 0.24^{cs}	2.73 ± 0.34^{ds}	3.90 ± 0.43^{cs}	4.06 ± 0.24^{as}
10 - 60	0.22 ± 0.08^{br}	0.94 ± 0.18^{cv}	2.19 ± 0.24^{dtu}	3.78 ± 0.61^{ct}	4.76 ± 0.12^{au}	4.93 ± 0.50^{at}
10 - 80	0.30 ± 0.09^{br}	1.75 ± 0.13^{bvx}	2.11 ± 0.42^{btu}	3.49 ± 0.54^{abt}	5.58 ± 0.13^{av}	5.57 ± 0.21^{au}
18 - 40	0.28 ± 0.07^{cr}	0.42 ± 0.17^{cs}	2.04 ± 0.27^{bt}	3.74 ± 0.12^{at}	4.43 ± 0.23^{at}	4.59 ± 0.17^{as}
18 - 60	0.33 ± 0.18^{br}	1.40 ± 0.17^{cw}	2.62 ± 0.19^{du}	4.36 ± 0.21^{et}	5.93 ± 0.47^{fv}	5.93 ± 0.60^{av}
18 - 80	0.39 ± 0.15^{br}	1.74 ± 0.33^{cr}	3.62 ± 0.11^{dxy}	6.29 ± 0.46^{eu}	7.43 ± 0.27^{fw}	7.61 ± 0.51^{aw}
26 - 40	0.27 ± 0.13^{ar}	0.82 ± 0.14^{cu}	3.33 ± 0.14^{ds}	4.60 ± 0.18^{adt}	5.98 ± 0.34^{av}	5.54 ± 0.11^{adu}
26 - 60	0.37 ± 0.11^{br}	1.70 ± 0.10^{csw}	4.31 ± 0.18^{ary}	8.21 ± 0.21^{av}	8.76 ± 0.51^{ax}	8.14 ± 0.50^{ax}
26 - 80	0.40 ± 0.14^{br}	1.93 ± 0.30^{cr}	4.59 ± 0.36^{dr}	9.26 ± 0.21^{ar}	9.38 ± 0.28^{ar}	8.35 ± 0.10^{ar}
Dietary lipid	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Dietary carotenoid	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Interaction of dietary lipid and carotenoid	P>0.05	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01

[1]Results are mean values of triplicates determination ± standard deviation. Values in each column with different superscript (a-f) and in each row with different superscript (q-z) are significantly different (P<0.05) from one another, respectively. The flesh of char prior to experiment contained 0.11 ± 0.10 mg/kg (on dry basis) of carotenoids.

Appendix 4B. Total carotenoid content (mg/kg, on dry basis) in the flesh of Arctic char after given time pigmentation[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
18 - 0	0.83 ± 0.18^{aq}	1.02 ± 0.12^{aq}	1.63 ± 0.76^{aq}	1.87 ± 0.33^{aq}	0.86 ± 0.15^{aq}	0.95 ± 0.11^{aq}
10 - 40	1.17 ± 0.52^{br}	1.61 ± 0.36^{bs}	5.83 ± 1.05^{es}	10.14 ± 0.34^{ds}	13.05 ± 0.17^{cs}	13.91 ± 0.27^{as}
10 - 60	1.05 ± 0.51^{br}	4.38 ± 0.28^{cv}	8.40 ± 0.97^{dtu}	13.66 ± 0.26^{ct}	16.15 ± 0.46^{au}	16.46 ± 0.18^{at}
10 - 80	1.43 ± 0.70^{br}	7.59 ± 0.52^{brx}	8.40 ± 1.74^{btu}	12.60 ± 0.26^{abt}	18.57 ± 0.50^{av}	18.58 ± 0.75^{au}
18 - 40	1.30 ± 0.62^{cr}	1.95 ± 0.33^{cs}	7.77 ± 1.05^{bt}	13.30 ± 0.24^{at}	14.48 ± 0.87^{at}	14.71 ± 0.58^{as}
18 - 60	1.60 ± 0.34^{br}	6.37 ± 0.81^{cw}	9.99 ± 0.77^{du}	15.07 ± 0.64^{et}	18.68 ± 0.17^{fv}	19.71 ± 0.52^{av}
18 - 80	1.83 ± 0.37^{br}	8.15 ± 0.40^{cr}	13.75 ± 0.43^{dxy}	22.16 ± 0.55^{eu}	23.73 ± 0.10^{fw}	24.32 ± 0.17^{aw}
26 - 40	1.31 ± 0.48^{br}	3.47 ± 0.18^{cu}	12.39 ± 0.55^{dx}	15.06 ± 1.22^{adt}	17.72 ± 0.12^{av}	17.24 ± 0.37^{adu}
26 - 60	1.73 ± 0.44^{br}	7.08 ± 0.44^{cxw}	15.00 ± 0.68^{ary}	26.64 ± 2.64^{av}	25.31 ± 0.17^{ax}	24.74 ± 0.16^{ax}
26 - 80	1.96 ± 0.94^{br}	8.21 ± 0.13^{cr}	15.97 ± 0.27^{dr}	29.44 ± 2.85^{ar}	27.95 ± 0.97^{ar}	25.91 ± 0.34^{ar}
Dietary lipid	$P>0.05$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
Dietary carotenoid	$P>0.05$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
Interaction of dietary lipid and carotenoid	$P>0.05$	$P<0.01$	$P<0.05$	$P<0.01$	$P<0.01$	$P<0.01$

[1]Results are mean values of triplicates determination ± standard deviation. Values in each column with different superscript (a-f) and in each row with different superscript (q-z) are significantly different ($P<0.05$) from one another, respectively. The flesh of char prior to experiment contained 0.57 ± 0.11 mg/kg (on dry basis) of carotenoids.

Appendix 5A. Total carotenoid content (mg/kg, on wet basis) in the belly skin of Arctic char after given time pigmentation[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
18 - 0	0.17 ± 0.05^{aq}	0.22 ± 0.09^{aq}	0.07 ± 0.09^{aq}	0.31 ± 0.07^{aq}	0.29 ± 0.04^{aq}	0.46 ± 0.13^{aq}
10 - 40	0.86 ± 0.21^{br}	1.17 ± 0.13^{cs}	2.35 ± 0.40^{ds}	5.67 ± 0.23^{es}	8.74 ± 0.12^{fs}	13.18 ± 0.13^{as}
10 - 60	0.96 ± 0.22^{br}	1.29 ± 0.28^{bs}	4.21 ± 0.47^{cu}	7.42 ± 0.11^{dt}	12.67 ± 0.16^{eu}	15.77 ± 0.12^{au}
10 - 80	1.04 ± 0.11^{br}	1.70 ± 0.33^{bst}	6.71 ± 0.35^{cy}	10.21 ± 016^{du}	15.69 ± 0.33^{ex}	13.80 ± 0.16^{ax}
18 - 40	1.01 ± 0.13^{br}	1.17 ± 0.21^{cst}	3.83 ± 0.37^{dt}	8.68 ± 0.60^{eu}	12.17 ± 0.11^{ft}	16.10 ± 0.28^{at}
18 - 60	1.04 ± 0.23^{br}	1.36 ± 0.13^{cst}	5.37 ± 0.27^{dw}	8.96 ± 0.88^{ev}	19.56 ± 0.40^{fw}	25.37 ± 0.54^{aw}
18 - 80	1.08 ± 0.27^{br}	1.25 ± 0.29^{bt}	9.25 ± 0.25^{cr}	16.35 ± 0.51^{dx}	21.09 ± 0.77^{ey}	30.92 ± 0.25^{ay}
26 - 40	0.92 ± 0.30^{br}	1.31 ± 0.14^{cst}	5.33 ± 0.17^{dv}	11.19 ± 0.10^{ev}	13.33 ± 0.25^{fv}	20.38 ± 0.79^{av}
26 - 60	0.91 ± 0.19^{br}	2.13 ± 0.17^{cu}	7.60 ± 0.41^{dx}	15.36 ± 0.12^{ew}	20.55 ± 0.63^{fz}	26.38 ± 0.17^{az}
26 - 80	1.03 ± 0.27^{br}	2.17 ± 0.27^{cr}	9.04 ± 0.59^{dz}	18.95 ± 0.30^{er}	25.97 ± 0.20^{fr}	32.47 ± 0.39^{ar}
Dietary lipid	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Dietary carotenoid	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Interaction of dietary lipid and carotenoid	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

[1]Results are mean values of triplicates determination ± standard deviation. Values in each column with different superscript (a-f) and in each row with different superscript (q-z) are significantly different (P<0.05) from one another, respectively. The belly skin of char prior to experiment contained 0.22 ± 0.05 mg/kg (on wet basis) of carotenoids.

Appendix 5B. Total carotenoid content (mg/kg, on dry basis) in the belly skin of Arctic char after given time pigmentation[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
18 - 0	0.62 ± 0.40^{aq}	0.69 ± 0.40^{aq}	0.22 ± 0.28^{aq}	0.62 ± 0.15^{aq}	0.71 ± 0.18^{aq}	0.99 ± 0.11^{aq}
10 - 40	2.88 ± 0.13^{br}	3.54 ± 0.14^{cs}	5.65 ± 0.50^{ds}	12.17 ± 0.95^{es}	24.44 ± 0.19^{fs}	33.45 ± 0.26^{as}
10 - 60	3.20 ± 0.14^{br}	3.64 ± 0.36^{bs}	9.92 ± 0.21^{cu}	13.90 ± 1.11^{dt}	30.71 ± 0.19^{eu}	37.99 ± 0.32^{au}
10 - 80	3.42 ± 0.83^{br}	4.32 ± 0.14^{bst}	16.24 ± 0.84^{cy}	19.33 ± 0.31^{du}	40.03 ± 0.50^{ex}	42.16 ± 0.40^{ax}
18 - 40	3.28 ± 0.23^{br}	3.83 ± 0.15^{cst}	8.76 ± 0.84^{dt}	18.33 ± 0.21^{eu}	27.90 ± 0.63^{ft}	45.26 ± 0.80^{at}
18 - 60	3.37 ± 0.10^{br}	4.10 ± 0.26^{cst}	12.45 ± 0.13^{dw}	21.27 ± 0.77^{ev}	38.08 ± 0.32^{fw}	69.40 ± 2.24^{aw}
18 - 80	3.83 ± 0.39^{br}	4.49 ± 0.42^{bt}	19.84 ± 0.26^{cr}	32.39 ± 0.16^{dx}	43.31 ± 0.10^{ey}	84.44 ± 0.67^{ay}
26 - 40	3.19 ± 0.38^{br}	4.15 ± 0.51^{cst}	10.94 ± 0.34^{dv}	22.07 ± 0.15^{ev}	33.19 ± 0.16^{fv}	51.89 ± 0.51^{av}
26 - 60	3.32 ± 0.17^{br}	6.23 ± 0.45^{cu}	14.45 ± 0.12^{dx}	24.92 ± 0.41^{ew}	48.79 ± 0.53^{fz}	77.43 ± 1.13^{az}
26 - 80	3.94 ± 0.11^{br}	7.28 ± 0.18^{cr}	18.59 ± 0.77^{dz}	33.54 ± 0.62^{er}	53.93 ± 0.20^{fr}	92.99 ± 0.20^{ar}
Dietary lipid	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Dietary carotenoid	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Interaction of dietary lipid and carotenoid	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

[1]Results are mean values of triplicates determination ± standard deviation. Values in each column with different superscript (a-f) and in each row with different superscript (q-z) are significantly different (P<0.05) from one another, respectively. The belly skin of char prior to experiment contained 0.78 ± 0.19 mg/kg (on dry basis) of carotenoids.

Appendix 6. Hunter-Colormet color scale L, a, b value of the fillet of Arctic char after given time pigmentation[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
L						
18 - 0	50.38 ±1.74	48.43 ± 2.44	48.18 ± 2.27	48.92 ± 3.68	47.88 ± 3.39	49.69 ± 2.06
10 - 40	48.98 ± 3.15	47.31 ± 2.07	46.81 ± 3.34	43.31 ± 2.02	44.39 ± 2.19	44.79 ± 1.51
10 - 60	46.74 ± 1.58	45.12 ± 2.31	44.13 ± 2.39	44.12 ± 1.42	41.57 ± 1.57	43.18 ± 2.59
10 - 80	45.98 ±1.22	42.34 ± 1.22	43.47 ± 2.27	42.26 ± 1.81	40.80 ± 0.45	39.40 ± 1.10
18 - 40	48.57 ± 1.40	46.48 ± 1.09	44.35 ± 3.19	43.31 ± 2.55	41.83 ± 0.85	41.62 ± 0.52
18 - 60	46.03 ± 0.74	46.16 ± 1.08	43.90 ± 3.11	40.29 ± 1.41	41.92 ± 1.04	40.27 ± 0.97
18 - 80	46.76 ± 2.17	45.47 ± 1.35	43.91 ± 3.68	43.57 ± 2.19	41.21 ± 3.31	41.61 ± 1.70
26 - 40	48.85 ± 3.48	46.65 ± 3.62	46.40 ± 1.98	46.32 ± 0.92	44.70 ± 1.60	43.54 ± 3.14
26 - 60	47.88 ± 2.15	46.10 ± 1.42	44.24 ± 1.94	41.30 ± 2.23	41.58 ± 2.58	42.19 ± 2.13
26 - 80	46.93 ± 2.24	45.84 ± 2.23	43.3 ± 2.66	40.08 ± 1.96	40.27 ± 3.07	41.80 ± 2.43
Dietary lipid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Dietary carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Interaction of dietary lipid and carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

[1]Results are mean values of 48 determinations ± standard deviation.

Appendix 6. Hunter-Colormet color scale L, a, b value of the fillet of Arctic char after given time pigmentation (continuted I)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
a						
18 - 0	0.28 ± 0.02^{at}	0.53 ± 0.07^{ar}	0.60 ± 0.02^{au}	0.54 ± 0.14^{au}	1.19 ± 0.53^{au}	1.21 ± 0.86^{at}
10 - 40	0.69 ± 0.10^{brs}	1.69 ± 0.62^{cr}	6.94 ± 1.32^{ds}	11.94 ± 1.67^{as}	14.03 ± 1.43^{as}	15.14 ± 1.01^{as}
10 - 60	1.08 ± 0.14^{brs}	2.82 ± 0.96^{cr}	7.29 ± 1.70^{dst}	12.72 ± 1.70^{cs}	14.56 ± 1.21^{acs}	16.04 ± 1.33^{ars}
10 - 80	1.39 ± 0.11^{brs}	2.87 ± 0.44^{cr}	8.23 ± 1.28^{dst}	13.84 ± 1.71^{ast}	15.80 ± 1.53^{ast}	17.25 ± 1.20^{ars}
18 - 40	0.99 ± 0.20^{brs}	1.67 ± 0.10^{cr}	6.01 ± 1.63^{ds}	14.68 ± 1.61^{ast}	16.79 ± 1.85^{arst}	16.86 ± 1.80^{ars}
18 - 60	1.08 ± 0.15^{brs}	2.59 ± 0.35^{cr}	7.42 ± 1.60^{ast}	16.30 ± 1.53^{ast}	18.07 ± 1.56^{arst}	18.37 ± 1.25^{ars}
18 - 80	1.48 ± 0.18^{br}	2.97 ± 0.64^{ar}	7.86 ± 1.21^{ast}	18.64 ± 2.34^{art}	19.30 ± 1.23^{art}	19.52 ± 2.83^{ars}
26 - 40	0.53 ± 0.08^{bs}	1.68 ± 0.05^{cr}	8.93 ± 1.27^{arst}	14.68 ± 2.58^{ast}	17.28 ± 1.77^{arst}	17.56 ± 2.09^{ars}
26 - 60	0.98 ± 0.08^{brs}	3.03 ± 0.90^{ar}	11.92 ± 2.01^{ar}	18.42 ± 2.40^{art}	18.87 ± 1.95^{art}	18.73 ± 1.76^{ars}
26 - 80	1.35 ± 0.58^{brs}	3.18 ± 0.75^{cr}	11.28 ± 1.91^{drt}	21.59 ± 0.90^{ar}	20.00 ± 1.25^{ar}	20.49 ± 2.31^{ar}
Dietary lipid	P>0.05	P>0.05	P<0.01	P<0.01	P<0.01	P<0.05
Dietary carotenoid	P<0.05	P<0.01	P<0.05	P<0.01	P<0.05	P<0.05
Interaction of dietary lipid and carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

[1]Results are mean values of 48 determinations ± standard deviation. Values in each row with different superscript (a-e) and in each column with different superscript (r-u) are significantly different (P<0.05) from one another, respectively.

Appendix 6. Hunter-Colormet color scale L, a, b value of the fillet of Arctic char after given time pigmentation (continuted II)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
b						
18 - 0	12.33 ± 1.07^{ar}	12.48 ± 1.50^{ar}	11.93 ± 1.37^{at}	11.19 ± 0.99^{at}	12.67 ± 0.94^{at}	12.39 ± 0.82^{at}
10 - 40	11.26 ± 1.14^{br}	14.44 ± 0.98^{abr}	14.62 ± 1.75^{abs}	19.94 ± 3.07^{as}	22.01 ± 1.19^{as}	21.61 ± 1.30^{as}
10 - 60	12.79 ± 1.61^{at}	13.17 ± 0.89^{ar}	15.64 ± 2.00^{ars}	20.52 ± 2.45^{as}	22.37 ± 1.34^{as}	22.16 ± 1.57^{ast}
10 - 80	12.31 ± 1.99^{br}	15.75 ± 1.48^{abr}	16.80 ± 2.07^{abrs}	21.26 ± 2.21^{as}	23.89 ± 1.86^{ars}	23.09 ± 1.03^{ars}
18 - 40	13.23 ± 0.70^{at}	13.53 ± 1.60^{ar}	15.51 ± 2.37^{ars}	21.89 ± 2.11^{ars}	22.68 ± 2.88^{as}	23.05 ± 1.93^{ars}
18 - 60	13.39 ± 2.55^{at}	13.68 ± 1.21^{ar}	16.21 ± 2.04^{ars}	22.24 ± 1.76^{ars}	24.99 ± 2.12^{ars}	23.99 ± 0.93^{ars}
18 - 80	15.34 ± 1.29^{at}	15.84 ± 2.20^{ar}	16.88 ± 1.72^{ars}	25.17 ± 2.72^{ars}	25.73 ± 1.23^{ars}	25.03 ± 1.30^{ars}
26 - 40	11.46 ± 2.31^{at}	13.64 ± 1.20^{ar}	16.27 ± 1.77^{ars}	22.49 ± 1.94^{ars}	23.05 ± 2.07^{ar}	23.86 ± 1.86^{ars}
26 - 60	11.93 ± 0.97^{br}	16.62 ± 1.99^{ar}	18.48 ± 1.29^{ars}	24.21 ± 2.29^{ars}	25.95 ± 1.68^{ars}	25.90 ± 1.17^{art}
26 - 80	14.00 ± 1.35^{br}	15.91 ± 0.95^{br}	20.37 ± 1.85^{ar}	27.81 ± 2.44^{ar}	27.64 ± 1.21^{ar}	26.16 ± 1.46^{ar}
Dietary lipid	$P>0.05$	$P>0.05$	$P<0.05$	$P<0.01$	$P<0.05$	$P<0.01$
Dietary carotenoid	$P>0.05$	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.01$	$P<0.05$
Interaction of dietary lipid and carotenoid	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$

[1]Results are mean values of 48 determinations ± standard deviation. Values in each row with different superscript (a,b) and in each column with different superscript (r-t) are significantly different ($P<0.05$) from one another, respectively.

Appendix 7. Hunter-Colormet color scale L, a, b value of the homogenized flesh of Arctic char after given time pigmentation[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
L						
18 - 0	70.69 ± 1.15^{ar}	68.98 ± 1.45^{ar}	66.60 ± 0.80^{abt}	66.42 ± 0.89^{arb}	65.83 ± 1.52^{abt}	65.33 ± 2.00^{bv}
10 - 40	68.60 ± 1.51^{ar}	68.58 ± 0.80^{ar}	65.22 ± 1.73^{ac}	63.07 ± 0.98^{cr}	62.08 ± 1.26^{cr}	58.96 ± 0.50^{br}
10 - 60	68.63 ± 1.12^{ar}	67.18 ± 1.64^{ar}	64.34 ± 1.27^{ar}	60.85 ± 1.21^{cr}	59.06 ± 0.46^{brs}	58.30 ± 0.44^{brt}
10 - 80	68.25 ± 0.60^{ar}	66.84 ± 2.20^{ar}	63.54 ± 0.74^{ar}	61.58 ± 0.62^{dr}	59.40 ± 1.38^{crs}	55.07 ± 0.67^{bs}
18 - 40	68.93 ± 0.81^{ar}	68.44 ± 1.05^{ar}	65.05 ± 1.03^{ar}	62.53 ± 0.86^{dr}	61.40 ± 0.25^{crs}	57.33 ± 0.67^{bt}
18 - 60	68.13 ± 1.13^{ar}	65.56 ± 1.03^{cr}	63.59 ± 2.03^{cr}	61.00 ± 1.02^{dr}	59.13 ± 0.95^{drs}	56.03 ± 0.66^{bsu}
18 - 80	68.55 ± 1.41^{ar}	65.90 ± 1.15^{cr}	64.60 ± 1.55^{cr}	61.69 ± 1.47^{cr}	58.56 ± 0.60^{bs}	55.20 ± 0.45^{bs}
26 - 40	68.63 ± 1.00^{ar}	68.24 ± 0.56^{cr}	64.56 ± 1.10^{cr}	61.41 ± 0.68^{dr}	60.25 ± 1.79^{drs}	57.59 ± 0.37^{bt}
26 - 60	68.31 ± 0.43^{ar}	65.95 ± 1.21^{cr}	64.31 ± 1.10^{cr}	61.30 ± 1.23^{br}	60.22 ± 1.68^{brs}	57.54 ± 0.55^{bt}
26 - 80	68.68 ± 1.07^{ar}	66.98 ± 0.59^{cr}	63.63 ± 1.41^{dr}	60.72 ± 1.76^{der}	58.40 ± 0.66^{es}	56.74 ± 0.64^{btu}
Dietary lipid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P<0.01
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<0.01

[1]Results are mean values of 12 determinations ± standard deviation. Values in each row with different superscript (a-e) and in each column with different superscript (r-v) are significantly different (P<0.05) from one another, respectively.

Appendix 7. Hunter-Colormet color scale L, a, b value of the homogenized flesh of Arctic char after given time pigmentation (continuted I)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	8		12	16	20	24
a						
18 - 0	0.08 ± 0.38^{av}	0.10 ± 0.90^{au}	0.06 ± 3.89^{av}	0.27 ± 0.25^{av}	0.13 ± 0.32^{au}	0.49 ± 0.40^{ax}
10 - 40	0.75 ± 0.77^{bau}	0.93 ± 0.21^{bx}	4.50 ± 0.71^{bx}	6.68 ± 0.68^{bx}	9.65 ± 1.17^{bx}	10.50 ± 0.61^{aw}
10 - 60	0.85 ± 0.11^{bat}	1.57 ± 0.06^{cat}	4.45 ± 0.55^{dx}	9.88 ± 0.52^{atu}	11.7 ± 0.74^{at}	11.71 ± 0.12^{av}
10 - 80	1.51 ± 0.17^{bax}	2.26 ± 0.38^{cat}	6.25 ± 0.18^{dtu}	10.95 ± 1.11^{at}	12.04 ± 0.87^{at}	12.32 ± 0.44^{atv}
18 - 40	1.03 ± 0.17^{bat}	1.91 ± 0.55^{bt}	5.04 ± 0.71^{csu}	10.91 ± 0.96^{dt}	11.69 ± 0.51^{at}	12.51 ± 0.27^{au}
18 - 60	1.43 ± 0.13^{bat}	2.03 ± 0.21^{bt}	5.23 ± 0.36^{csu}	11.30 ± 0.50^{dtu}	12.27 ± 0.30^{ct}	$14.01 \pm 0.33as$
18 - 80	1.73 ± 0.37^{bt}	2.58 ± 0.43^{cax}	6.73 ± 0.52^{dt}	12.78 ± 0.61^{ct}	13.48 ± 0.54^{acat}	14.36 ± 0.68^{astx}
26 - 40	0.64 ± 0.15^{btu}	1.18 ± 0.18^{cx}	5.78 ± 0.16^{dtu}	9.10 ± 0.51^{at}	12.30 ± 1.25^{at}	13.26 ± 0.97^{astu}
26 - 60	0.88 ± 0.09^{bat}	2.10 ± 0.37^{bt}	6.83 ± 0.68^{cat}	12.94 ± 1.13^{dt}	12.72 ± 0.29^{ct}	14.40 ± 0.54^{as}
26 - 80	1.58 ± 0.16^{bax}	2.98 ± 0.43^{ct}	7.44 ± 0.40^{dt}	16.00 ± 0.37^{et}	14.71 ± 0.45^{ft}	15.98 ± 0.48^{at}
Dietary lipid	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Dietary carotenoid	P<0.05	P<0.01	P<0.01	P<0.01	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<0.01	P<0.01

[1]Results are mean values of 12 determinations ± standard deviation. Values in each row with different superscript (a-f) and in each column with different superscript (r-x) are significantly different (P<0.05) from one another, respectively.

Appendix 7. Hunter-Colormet color scale L, a, b value of the homogenized flesh of Arctic char after given time pigmentation (continuted II)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
b						
18 - 0	12.18 ± 0.24^{bu}	12.75 ± 1.46^{bu}	14.25 ± 0.28^{abt}	14.51 ± 0.31^{av}	12.66 ± 0.79^{abu}	13.24 ± 0.69^{abt}
10 - 40	12.76 ± 0.71^{bst}	14.08 ± 0.46^{cs}	16.78 ± 0.25^{ds}	20.13 ± 0.46^{as}	20.86 ± 1.13^{as}	20.95 ± 0.61^{as}
10 - 60	13.70 ± 0.36^{bs}	15.43 ± 0.31^{crt}	17.50 ± 0.87^{as}	21.18 ± 0.66^{ast}	22.36 ± 0.35^{ast}	22.70 ± 1.31^{as}
10 - 80	15.14 ± 0.14^{bt}	16.34 ± 0.44^{at}	17.61 ± 0.71^{as}	20.32 ± 0.57^{artu}	23.69 ± 1.07^{at}	23.70 ± 0.85^{as}
18 - 40	12.28 ± 0.15^{bt}	13.80 ± 0.08^{cs}	17.27 ± 0.63^{ds}	21.56 ± 0.77^{adsu}	21.39 ± 1.00^{adst}	22.69 ± 0.39^{as}
18 - 60	13.23 ± 0.87^{bst}	15.76 ± 0.38^{crt}	18.01 ± 0.65^{ds}	22.72 ± 0.31^{ctu}	23.78 ± 0.62^{at}	24.68 ± 1.17^{ats}
18 - 80	14.69 ± 0.52^{bt}	15.96 ± 0.35^{crt}	19.29 ± 0.41^{dt}	24.70 ± 0.58^{at}	24.93 ± 0.79^{at}	25.06 ± 0.95^{ats}
26 - 40	12.28 ± 0.39^{bt}	14.90 ± 0.36^{ct}	19.35 ± 1.00^{dt}	21.96 ± 0.78^{adtu}	22.70 ± 1.79^{adst}	23.09 ± 0.52^{ats}
26 - 60	13.03 ± 0.32^{bst}	15.93 ± 0.57^{crt}	19.91 ± 0.35^{dt}	25.76 ± 1.46^{adrt}	25.30 ± 0.56^{adt}	25.44 ± 0.29^{ats}
26 - 80	14.93 ± 0.17^{bt}	16.28 ± 0.78^{crt}	20.10 ± 0.48^{dt}	26.44 ± 0.80^{art}	26.33 ± 0.94^{at}	25.95 ± 0.43^{at}
Dietary lipid	P>0.05	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01
Dietary carotenoid	P<0.01	P<0.01	P<0.01	P<0.01	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

[1]Results are mean values of 12 determinations ± standard deviation. Values in each row with different superscript (a-d) and in each column with different superscript (r-v) are significantly different (P<0.05) from one another, respectively.

Appendix 8. Hunter-Colormet color scale L, a, b value of the belly skin of Arctic char after given time pigmentation[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
L						
18 - 0	81.06 ± 1.58	77.32 ± 3.28	76.06 ± 2.03	75.90 ± 2.52	75.02 ± 2.25	72.05 ± 1.84
10 - 40	82.09 ± 1.89	78.03 ± 2.89	76.82 ± 2.26	76.51 ± 8.21	66.05 ± 6.37	67.27 ± 5.66
10 - 60	76.96 ± 4.46	76.54 ± 3.22	73.18 ± 6.38	69.37 ± 6.33	69.37 ± 4.71	62.40 ± 2.57
10 - 80	78.14 ± 3.49	75.97 ± 2.48	74.18 ± 3.54	74.18 ± 1.87	66.13 ± 3.90	63.96 ± 3.99
18 - 40	79.10 ± 3.87	78.14 ± 3.44	76.33 ± 3.37	73.96 ± 4.13	68.82 ± 4.96	66.23 ± 2.58
18 - 60	81.98 ± 2.86	74.46 ± 2.99	74.55 ± 1.36	72.15 ± 4.68	69.15 ± 6.59	65.15 ± 3.84
18 - 80	79.18 ± 2.60	74.64 ± 1.68	74.16 ± 3.89	73.00 ± 2.27	66.27 ± 6.79	62.00 ± 6.37
26 - 40	78.95 ± 1.78	74.13 ± 1.65	72.93 ± 4.33	72.34 ± 6.74	65.95 ± 6.16	65.84 ± 3.77
26 - 60	76.33 ± 1.43	73.62 ± 4.34	72.93 ± 4.04	70.96 ± 3.37	66.75 ± 4.79	63.54 ± 5.57
26 - 80	76.42 ± 2.46	76.07 ± 3.62	73.76 ± 3.39	73.86 ± 2.10	68.94 ± 4.23	62.86 ± 5.44
Dietary lipid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Dietary carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Interaction of dietary lipid and carotenoid	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

[1]Results are mean values of 48 determinations ± standard deviation.

Appendix 8. Hunter-Colormet color scale L, a, b value of the belly skin of Arctic char after given time pigmentation (continuted I)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
a						
18 - 0	-1.46 ± 0.28^{at}	-1.78 ± 0.83^{at}	-1.04 ± 0.63^{as}	-0.78 ± 0.69^{as}	-0.62 ± 1.15^{au}	-0.51 ± 0.98^{at}
10 - 40	-2.03 ± 1.23^{bt}	-2.44 ± 0.93^{bt}	-0.91 ± 0.85^{bs}	-0.68 ± 0.81^{bs}	4.90 ± 2.98^{as}	9.12 ± 2.44^{as}
10 - 60	-1.51 ± 0.99^{ct}	-0.91 ± 0.93^{cst}	0.66 ± 0.91^{cst}	4.51 ± 1.91^{bt}	5.73 ± 1.05^{bs}	12.34 ± 2.49^{as}
10 - 80	-3.18 ± 0.49^{dt}	-0.78 ± 0.89^{cst}	3.12 ± 1.98^{bst}	4.77 ± 1.82^{bt}	6.24 ± 1.57^{bs}	12.11 ± 2.60^{as}
18 - 40	-3.06 ± 0.69^{ct}	-1.97 ± 0.44^{ctu}	-0.47 ± 0.57^{bs}	5.22 ± 2.00^{at}	6.00 ± 2.92^{as}	9.63 ± 3.92^{as}
18 - 60	-3.01 ± 0.96^{dt}	0.07 ± 0.50^{cs}	4.10 ± 1.22^{bst}	6.20 ± 1.61^{bt}	7.30 ± 1.29^{bst}	11.90 ± 2.26^{as}
18 - 80	-3.13 ± 0.68^{et}	-0.39 ± 0.45^{dsu}	4.41 ± 1.41^{cst}	6.51 ± 1.88^{bcr}	9.66 ± 1.53^{bst}	16.62 ± 3.57^{as}
26 - 40	-2.68 ± 0.99^{ct}	0.36 ± 0.72^{bs}	3.06 ± 1.35^{abt}	4.27 ± 1.66^{abu}	12.16 ± 2.80^{at}	12.46 ± 2.52^{as}
26 - 60	-1.09 ± 0.60^{ct}	1.36 ± 0.80^{bcs}	4.89 ± 1.23^{as}	6.22 ± 1.22^{at}	14.00 ± 3.54^{art}	14.72 ± 2.60^{as}
26 - 80	-1.36 ± 0.44^{ct}	2.29 ± 1.20^{bt}	6.78 ± 1.24^{abt}	7.44 ± 1.85^{abt}	17.92 ± 2.34^{at}	19.44 ± 4.69^{at}
Dietary lipid	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05
Dietary carotenoid	P>0.05	P<0.01	P<0.01	P<0.01	P<0.05	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

[1]Results are mean values of 48 determinations ± standard deviation. Values in each row with different superscript (a-d) and in each column with different superscript (r-u) are significantly different (P<0.05) from one another, respectively.

Appendix 8. Hunter-Colormet color scale L, a, b value of the belly skin of Arctic char after given time pigmentation (continuted II)[1]

Diet(%lipid- ppm carotenoid)	Time interval (weeks)					
	4	8	12	16	20	24
b						
18 - 0	5.40 ± 1.42^{br}	5.66 ± 1.77^{ar}	7.91 ± 2.41^{ar}	8.62 ± 2.41^{as}	10.41 ± 2.72^{as}	11.52 ± 2.29^{as}
10 - 40	5.48 ± 1.00^{ar}	9.06 ± 2.17^{ar}	9.88 ± 2.21^{ar}	9.16 ± 2.13^{as}	10.03 ± 1.85^{as}	12.21 ± 1.04^{as}
10 - 60	6.18 ± 0.46^{ar}	7.90 ± 3.17^{ar}	11.55 ± 3.22^{ar}	11.63 ± 2.19^{ars}	13.05 ± 1.38^{ars}	14.31 ± 1.54^{ars}
10 - 80	7.54 ± 1.48^{ar}	10.20 ± 2.26^{ar}	11.65 ± 1.92^{ar}	14.78 ± 0.95^{ars}	14.22 ± 2.67^{ars}	15.52 ± 0.81^{ars}
18 - 40	5.86 ± 1.55^{ar}	7.36 ± 1.27^{ar}	7.73 ± 2.82^{ar}	12.08 ± 3.11^{ars}	12.90 ± 1.94^{ars}	13.07 ± 1.05^{ars}
18 - 60	6.66 ± 1.73^{ar}	9.29 ± 2.68^{ar}	11.59 ± 3.56^{ar}	12.61 ± 0.62^{ars}	13.60 ± 1.89^{ars}	16.82 ± 1.91^{ars}
18 - 80	6.86 ± 2.73^{br}	9.29 ± 1.52^{br}	12.33 ± 2.03^{ar}	14.17 ± 2.30^{ars}	15.61 ± 2.64^{ars}	18.70 ± 1.14^{ar}
26 - 40	7.40 ± 1.47^{ar}	9.21 ± 1.01^{ar}	12.96 ± 2.15^{ar}	14.32 ± 2.62^{ars}	14.01 ± 2.40^{ars}	14.68 ± 1.53^{ars}
26 - 60	7.80 ± 1.18^{ar}	10.53 ± 2.53^{ar}	13.91 ± 2.05^{ar}	14.82 ± 1.05^{ars}	16.48 ± 2.05^{ars}	17.43 ± 2.91^{ars}
26 - 80	8.31 ± 1.18^{ar}	10.58 ± 3.33^{ar}	14.26 ± 3.71^{ar}	15.92 ± 1.53^{ar}	17.33 ± 2.46^{ar}	19.10 ± 2.27^{ar}
Dietary lipid	P>0.05	P>0.05	P<0.05	P<0.01	P<0.05	P<0.05
Dietary carotenoid	P>0.05	P>0.05	P>0.05	P<0.01	P<0.05	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

[1]Results are mean values of 48 determinations ± standard deviation. Values in each row with different superscript (a,b) and in each column with different superscript (r,s) are significantly different (P<0.05) from one another, respectively.