

Carotenoid pigmentation of salmonid fishes - recent progress¹

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ABSTRACT: The purpose of this presentation is to review recent literature on carotenoid pigmentation in salmonid fishes. Consumers have preferences for red-colored products of salmonid fishes, and this is reflected by an increased rate of publications on carotenoids in relation to aspects of quality such as coloration, variation among groups of fish and within fish, and effects of fish production and processing. Recent studies have shown that dietary carotenoid utilization for muscle pigmentation has a considerable genetic component, enabling selective breeding for color traits. Recently, studies have shown that astaxanthin (3,3'-dihydroxy- β , β -carotene-4,4'-dione) *E/Z* isomers accumulate selectively in fish tissues and plasma. All-*E*-astaxanthin accumulate selectively in muscle and plasma, and 13*Z*-astaxanthin in liver of salmonid fishes. The positive effect of lipid content on muscle carotenoid deposition appear to be the only interaction with macronutrients. Also it has been shown that oil composition and α -tocopherol may have slight positive effects on carotenoid utilization. Storage losses of carotenoids may be considerable, and protection from oxygen is of great importance. Carotenoid absorption is slow, and apparently governed by passive diffusion. About 60% of the absorbed carotenoids are metabolized, partly into idoxanthin (3,3',4'-trihydroxy- β , β -carotene-4-one) which is rapidly formed from astaxanthin. Carotenoid metabolism and partitioning among body compartments is affected by sex hormones. Positive effects of astaxanthin on growth and disease resistance may be related to carotenoid antioxidant activity.

KEY WORDS: Carotenoids, Astaxanthin, Canthaxanthin, *Salmo*, *Oncorhynchus*, Color

INTRODUCTION

The conspicuous coloration of many seafoods is due to carotenoid pigments (Shahidi *et al.* 1998). Thus the coloration of important aquaculture species includes the flesh of salmonid fishes, exoskeleton and muscular epithelium of shrimps, lobster and other crustacean carapaces, integuments of red and yellow fishes, and molluscan gonads and hepatopancreas. In salmonid fishes fillet color is held to be an important quality parameter (Sigurgisladottir *et al.* 1997), and pigment feeding is regarded as the most important management practice for marketing of farmed salmon (Moe, 1990). Astaxanthin (3,3'-dihydroxy- β , β -carotene-4,4'-dione) and canthaxanthin (β , β -carotene-4,4'-dione), either alone or in combination, are the carotenoids most commonly used for pigmentation of salmonid fishes. Addition of carotenoids to animal diets is expensive, and usually less than 20% of the dietary carotenoids contribute to flesh pigmentation in salmonid fishes (Torrissen *et al.* 1989; Storebakken and No, 1992). Astaxanthin consists of three chiral *R/S* isomers, Fig. 1, and 272 possible geometrical *E/Z* isomers of which the quantitatively most important are shown in Fig. 2.

¹ Bjerkeng, B. 2000. Carotenoid pigmentation of salmonid fishes - recent progress. In: Cruz -Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A. y Civera-Cerecedo, R., (Eds.). Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola. 19-22 Noviembre, 2000. Mérida, Yucatán.

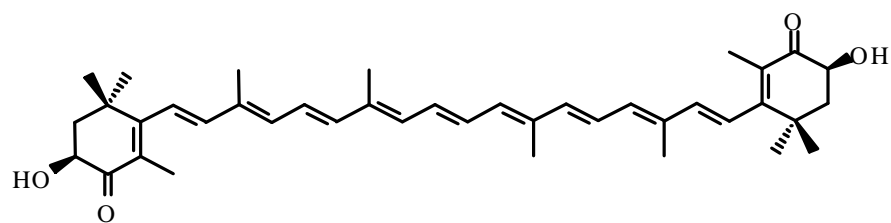
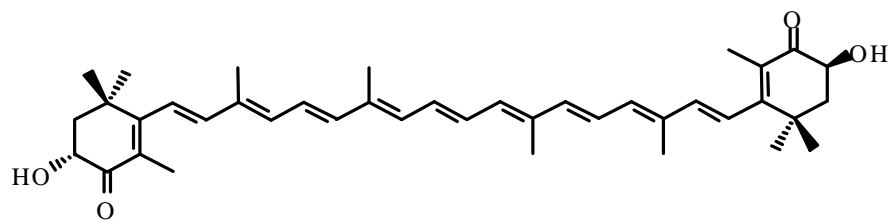
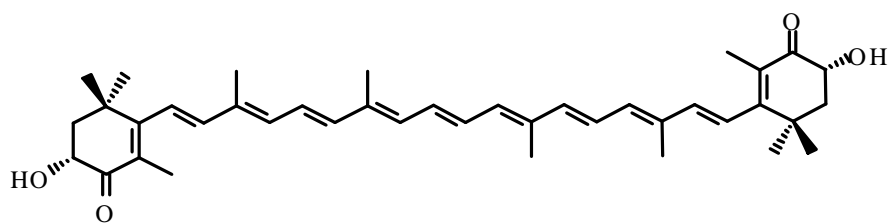
**1a****2a****3a**

Figure 1. Chemical structures of (3*S*,3'*S*)- **1a**, (3*R*,3'*S*)- **2a**, (3*R*,3'*R*) - astaxanthin **3a**.

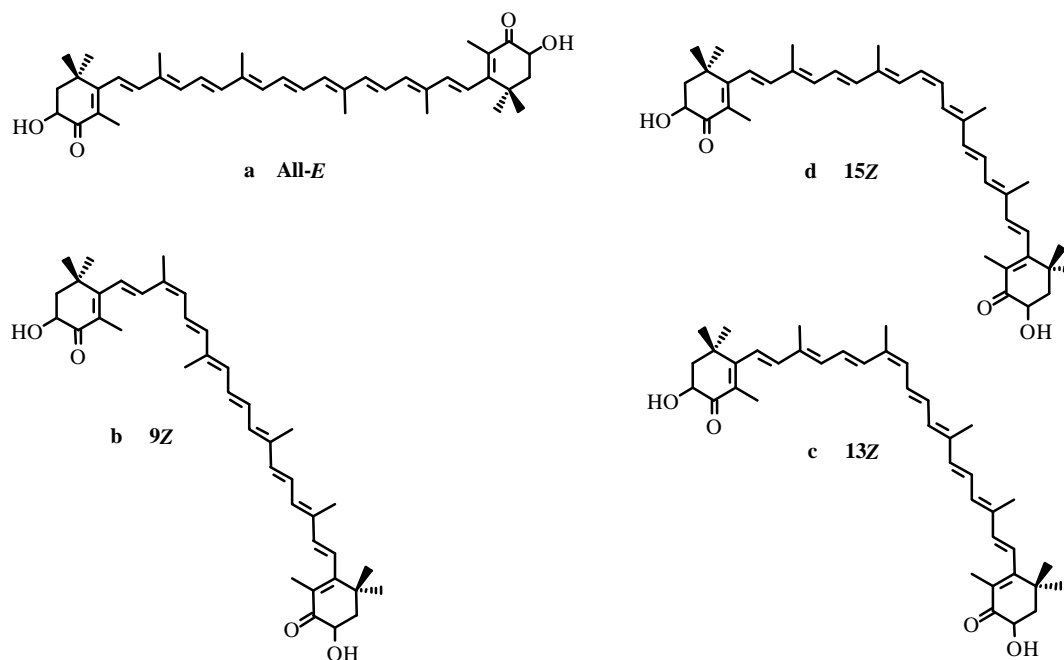


Figure 2. Structures of all-E-(1a, 2a,3a), 9Z-(1b, 2b,3b), 13Z-(1c, 2c,3c) and 15Z-astaxanthin (1d, 2d,3d).

Literature related to carotenoid pigmentation of aquaculture and seafood species is covered by several previous reviews (Simpson *et al.* 1981; Kantha, 1989; Matsuno and Hirao, 1989; Torrissen *et al.* 1989; Putnam, 1991; Storebakken and No, 1992; Meyers, 1994; Bjerkeng, 1997; Schiedt, 1998; Shahidi *et al.* 1998). The purpose of this presentation is to review recent literature on carotenoid pigmentation in salmonid fishes.

Importance of pigmentation in salmonid fishes

Consumers have preferences for red-colored products of salmonid fishes (Ostrander *et al.* 1976; Hatano *et al.* 1987; Gormley, 1992; Rounds *et al.* 1992; Sigurgisladottir *et al.* 1994; Skonberg *et al.* 1998). Redness contributes significantly to overall enjoyment of cooked salmonid flesh (Sylvia *et al.* 1995), and may have a signalling value as an indicator of product quality (Sylvia *et al.*, 1996). Addition of astaxanthin may be performed during processing of minced products of salmon muscle without negatively affecting product flavor (Østerlie *et al.* 2000), due to little effect of astaxanthin on flavor.

Astaxanthin is the most efficient carotenoid used for salmonid pigmentation (Torrissen *et al.* 1989; Storebakken and No, 1992), and is responsible for 15-20% of the total feed cost, or 6-8% of the total

Atlantic salmon, *Salmo salar*, production cost (Torrissen, 1995). The retention of dietary carotenoids by the muscle tissues varies from only 2 – 22 % in salmonid species (Torrissen *et al.* 1989; Storebakken and No, 1992). According to Torrissen and Christiansen (1995a), canthaxanthin or astaxanthin should be regarded as a vitamin for fish and all fish diets should be added with canthaxanthin or astaxanthin at a level above 10 mg/kg dry feed to ensure the well-being of the animal.

Appropriate flesh color determines acceptance and price of salmon products. Therefore, it is important to know how coloration and pigmentation may vary among individuals and groups of salmonid fish. The magnitude of these variations and the factors that govern them, along with the impact of slaughter, storage and processing on coloration and pigmentation is discussed.

Color

The canonical dimensions of perceived color space are hue, saturation and brightness. When colors are compared the human eye may distinguish about 10 million different color hues. However, the capacity to remember colors and magnitude of hue sensations is limited to about 300. Objective means for recording coloration are thus needed. Visual color assessments may be aided by the use of color charts such as the Roche Colour Card for Salmonids and the *SalmoFan* (Hoffmann-La Roche, Basel, Switzerland). Such assessments represent crude measures to characterize and classify pigmentation of fish flesh, primarily for the use by fish farmers, fish feed producers, processing industry and marketing organizations. The degree of precision in color determination is relatively low due to influences of illumination, individual abilities to distinguish colors, and qualities of the objects surface. Carotenoid concentration alone cannot be used as a criterion of perceived color (Little *et al.* 1979). This may be related to the lack of correlation between astaxanthin concentration and muscle fibre density, and the positive relationship between *SalmoFan* score and muscle fibre density (Johnston *et al.* 2000).

Coloration is determined by the specific carotenoids used and the carotenoid composition. Canthaxanthin pigmented farmed salmon is more yellowish than astaxanthin pigmented wild salmon (Skrede and Storebakken, 1986). Idoxanthin (3,3',4'-trihydroxy- β , β -carotene-4-one), an astaxanthin metabolite, may accumulate in considerable amounts in the muscle (up to 30% of total carotenoids in large Atlantic salmon; Schiedt, 1998, and 40-60% of total carotenoids in Arctic charr; Aas *et al.* 1997; Hatlen *et al.*, 1997; Bjerkeng *et al.* 2000a; trace amounts in rainbow trout Bjerkeng *et al.* 1997a). It has a shorter chromophore than astaxanthin, and appears as more yellow. Accumulation of idoxanthin in the muscle may therefore contribute to a more yellowish hue of the flesh as indicated by Hatlen *et al.* (1998).

The most frequently used instrumental color measures are the CIE [1976] L*, a*, b*, and the Hunter L,a,b systems, which primary parameters are lightness (L*), redness (a*) and yellowness (b*). Muscle coloration and the concentration of carotenoids in Atlantic salmon are significantly related, but when astaxanthin concentration exceeds about 8 mg/kg, the eye becomes saturated, the increase in color perception upon increase in pigment concentration levels off, and color hues cannot be distinguished accurately.

The a*-value generally exhibits the best correlation to increasing carotenoid levels. Several different mathematical models that describe the relationship between instrumentally and visually assessed coloration, and carotenoid concentration are found in the literature (cf. Hatlen *et al.* 1998). The models differ slightly, and are representations of the specific material presented by the various authors. One

reason may be age-related, manifested by increase in fibre-optic reflectance and decrease in color scores (Swatland *et al.* 1998). The response in the a^* -value diminishes at increasing carotenoid levels, and it becomes increasingly difficult to predict carotenoid concentrations with increasing a^* -values. This fact is illustrated by Wathne *et al.* (1998) data on individual Atlantic salmon, Fig. 3.

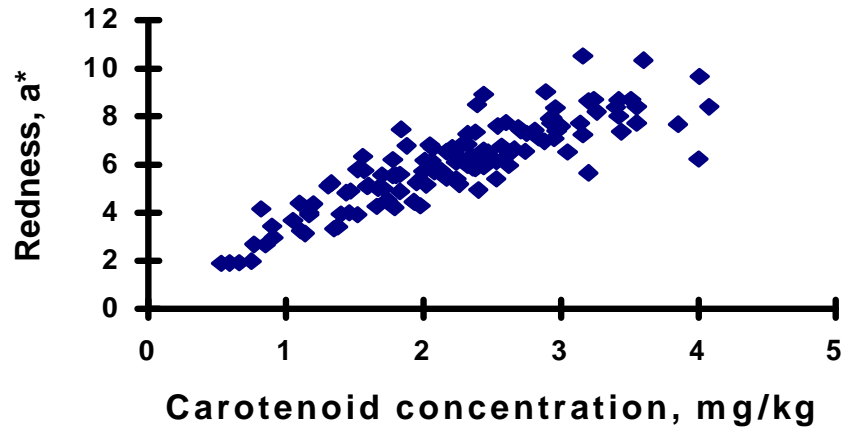


Figure 3. Relationship between redness (a^*) and carotenoid concentration (mg/kg) in Atlantic salmon fillets (Based on data from Wathne *et al.* 1998).

Several reports have recently shown that higher correlations may be achieved by employing non-linear relationships when the muscle carotenoid concentration is high. Thus, Christiansen *et al.* (1995a) found a high correlation between a^* , b^* and chroma (C_{ab}^*) and the logarithm of fillet carotenoid concentration in Atlantic salmon. Analogously, increased correlations have been obtained between the product of Hunter a and b colourimeter values, and carotenoid concentration in the fillet of rainbow trout, *Oncorhynchus mykiss* (King, 1996). Bjerkeng *et al.* (1997a) found that the logarithm of the a^* -values and the logarithm of fillet carotenoid concentration, and L^* -values negatively linearly with the fillet carotenoid concentration gave the highest correlation, Fig. 4. Fibre-optic spectrophotometry may be employed for robotic color-sorting of fish (Swatland *et al.* 1997).

Color parameters (CIE [1976] $L^*a^*b^*$) are also correlated to the fat content of the muscle (Einen and Skrede, 1998). At the same astaxanthin levels in muscle, fillet fat content contributes to higher values a^* and b^* , but a less red and more yellowish hue (h_{ab}). The use of tristimulus color parameters (CIE [1976] $L^*a^*b^*$) have limited use for prediction of carotenoid concentration when carotenoid concentrations are high (cf. Fig. 4). Therefore, their use in evaluation of carotenoid utilization in salmonids should be restricted to small fish, or fish containing less than ca. 5 mg astaxanthin/kg. The use of astaxanthin concentration as a parameter for astaxanthin utilization is much preferred.

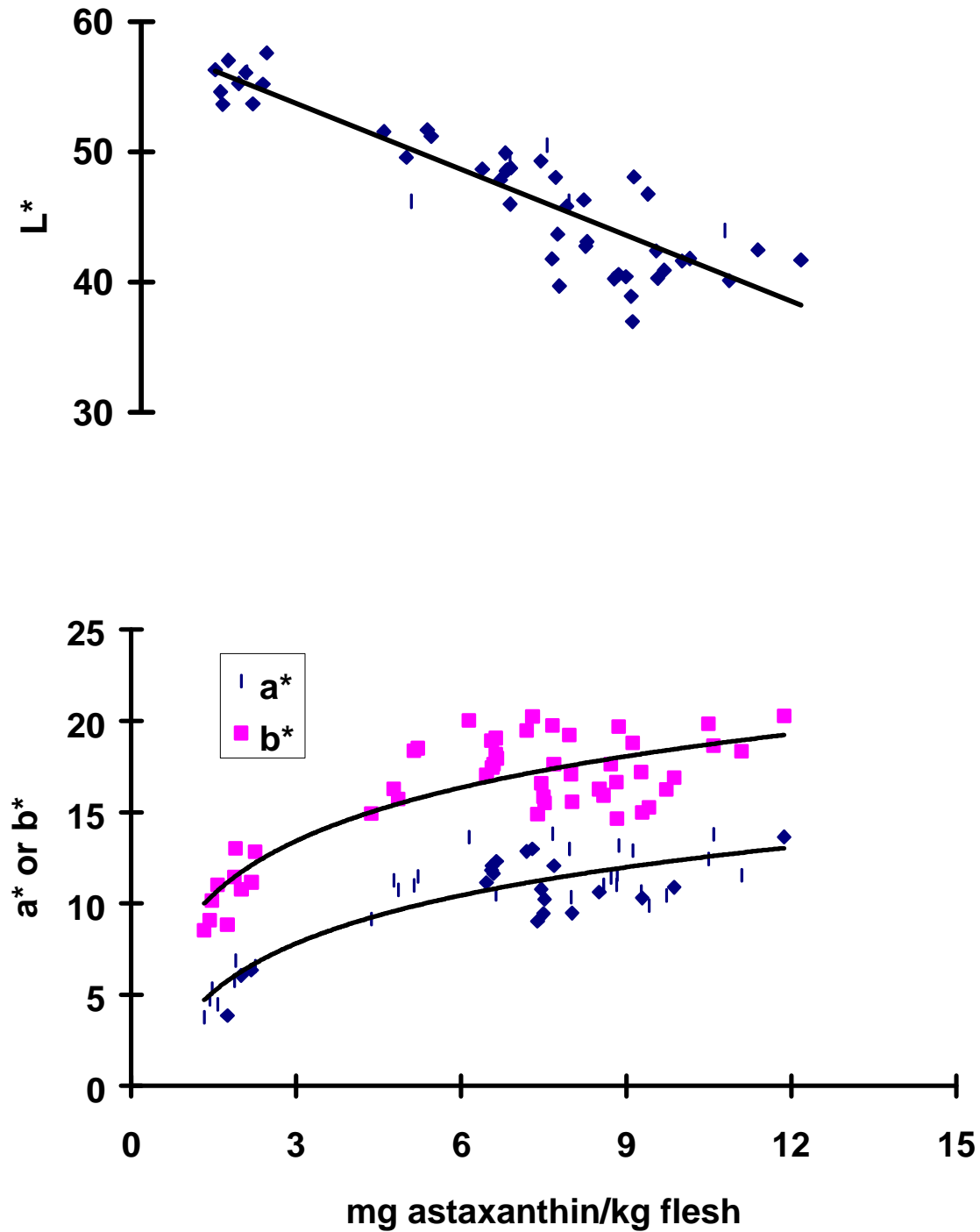


Figure 4. Relationship between redness (a^*), yellowness (b^*), lightness (L^*) and carotenoid concentration (mg/kg) of Atlantic salmon fillets (Based on data from Bjerkeng *et al.* 1997a).

Muscle carotenoid distribution

After smoltification muscle deposition of carotenoids increase (cf. Bjerkeng *et al.*, 1992), and accumulation of carotenoids do not cease until sexual maturation. The muscle tissues have relatively low astaxanthin concentrations but account for the largest body pool of astaxanthin. Astaxanthin is primarily associated with the muscle protein fraction in salmonids (reviewed by Schiedt, 1998), but is not evenly distributed within the fish fillets. This sets important constraints with respect to quality. A longitudinal variation in carotenoid content and red color is found, with more astaxanthin being deposited in the caudal than in the anterior part. The caudal part may contain 30-40% more carotenoids than the back and neck parts of the fillets (Christiansen and Wallace, 1988; No and Storebakken, 1991). Refsgaard *et al.* (1998a) reported a 19% difference in astaxanthin concentration in favor of the caudal part. This was not found by Bell *et al.* (1998). Also there is a radial increase in pigmentation towards the backbone (McCallum *et al.* 1987). The dorsal and midline cut areas contain more carotenoids than the adipose tissues in the belly flap and the part connecting the myotomes ("zebra-stripes"). Facile comparisons between results on carotenoids levels and pigmentation may be performed by using standardized sampling.

Phenotypic and genetic variation

Although relationships between fish size and pigment level are found, large individual variation within weight classes, even in fish fed the same pigment level and kept in the same cage may be expected. Selection of different weight classes may therefore accommodate markets and applications with different requirements for color and pigmentation. Knowledge and determination of variation in pigment levels and coloration with size is therefore important for selecting correct weight classes for such use.

We have made estimates of phenotypic and genetic parameters for pigmentation and coloration data based on 85 sib-groups of Atlantic salmon fed a diet with 100 mg astaxanthin kg⁻¹ (Bjerkeng *et al.*, 1996). The weight of the sampled fish (ca. 850) was normally distributed, the average weight being 4.7 kg. This was also true for concentration of astaxanthin and idoxanthin in the fillet and the CIE 1976 a*-values. The mean total carotenoid content of the fillets was 7.0 mg kg⁻¹, and the mean content of astaxanthin and idoxanthin was 96.0 and 2.9% of total carotenoids, respectively. The fillets of individual fish exhibited a wide range in total carotenoid content (from approximately 0.5-13.3 mg kg⁻¹), whereas the range for different families was 4.2-9.4 mg kg⁻¹. The idoxanthin content in the fillets was relatively low and family means ranged from 0.4-8.3% of total carotenoids. However, a relatively low correlation (0.41) between CIE a*-values and total carotenoid content (mg/kg) in the fillet was observed. This correlation is somewhat lower than the correlation (0.60) observed by Christiansen *et al.* (1995a), investigating Atlantic salmon within a concentration range of 0.5 to 10.5 mg carotenoids/kg. In rainbow trout there appears to be an innate variation of 20-25% in flesh pigmentation at a market weight of 400 g (Nickell and Bromage, 1998a). It should be noted that serum levels of carotenoids are poor indicators of muscle carotenoid levels (Gobantes *et al.* 1998a; Wathne *et al.* 1998).

Evaluations of fillet coloration and pigmentation in salmonids for determination of heritabilities and genetic correlations of related traits has been based on sensory assessed intensity scaling, either alone (Rye and Gjerde, 1996) or combined with color reference cards (Choubert *et al.* 1997). Also instrumentally measured color characteristics, CIE 1976 (L*a*b*), (Blanc and Choubert, 1993; Withler and Beacham, 1994; Choubert *et al.* 1997), or spectrophotometrical quantification based on total

carotenoid in extracts (Elvingson and Nilsson, 1994) have been used. These studies have revealed a significant genetic component in variation in pigmentation and coloration. Thus fish with improved pigmentation may be obtained through selective breeding (Gjedrem, 2000).

Bjerkeng *et al.* (1996) using HPLC determination of carotenoids, found substantial additive genetic variation for traits related to coloration and pigmentation, Table 1. Estimated heritabilities for color traits derived from the sire component of variance ranged from 0.24 ± 0.12 to 0.60 ± 0.18 . Corresponding estimates for body weight and fat content were 0.43 ± 0.18 and 0.35 ± 0.15 , respectively. Genetic correlations were low to intermediate in magnitude (-0.04 to 0.53), except for very high, positive correlations observed between a^* -values and astaxanthin content in muscle, and between astaxanthin contents in muscle and in plasma (>0.90). The standard errors of the estimated genetic correlations were all large (0.25 - 0.50). The positive genetic correlations between weight and astaxanthin and a^* value imply that selection for growth rate, indirectly contributes to increased astaxanthin content and a^* - value. On the other hand, selection for lower fat content may reduce the astaxanthin content. However, by using a selection index it is possible to obtain genetic gain in many quality traits simultaneously.

Table 1. Genetic correlations of some quality traits related to coloration and pigmentation in Atlantic salmon (based on Bjerkeng *et al.* 1996).

| Traits | Genetic correlation |
|--|---------------------|
| Weight-Astaxanthin in flesh | 0.34 ± 0.41 |
| Weight- a^* | 0.49 ± 0.26 |
| Fat- a^* | 0.08 ± 0.26 |
| Fat-Astaxanthin in flesh | 0.27 ± 0.52 |
| a^* -Astaxanthin in flesh | (1.20 ± 0.36) |
| Astaxanthin-Idoxanthin, % of total carotenoid, flesh | -0.61 ± 0.52 |

Dietary pigment sources

Carotenoid pigmentation is affected by dietary pigment source, dosage level, duration of feeding and by dietary composition. Astaxanthin sources for muscle pigmentation contain ca. 75% all-*E*-astaxanthin and 25% *Z*-astaxanthins of total astaxanthin. Recent evidence show that all-*E*-astaxanthin is utilized to a higher extent for flesh pigmentation than the *Z*-isomers, although the precise numerical differences are uncertain.

We have investigated the apparent digestibility coefficients (ADC), muscle accumulation (Bjerkeng *et al.* 1997a), and the relative concentrations in plasma, liver, skin, and kidney of astaxanthin *E/Z*-isomers (Østerlie *et al.* 1999) in rainbow trout (*Oncorhynchus mykiss*). The fish were fed diets containing either predominantly all-*E*-astaxanthin (97% of total astaxanthin) or a mixture of all-*E*- and *Z*-astaxanthins (64 and 36%, respectively). The ADC of total astaxanthin was significantly higher in trout fed all-*E*-astaxanthin (79%) compared to trout fed the *E/Z* mixture (64%). In decreasing order the following ADCs were found: all-*E*-astaxanthin > 13*Z*-astaxanthin > 9*Z*-astaxanthin. Accordingly, a higher carotenoid concentration was observed in plasma of trout fed diets with all-*E*-astaxanthin compared to trout fed the astaxanthin stereoisomer mixture, and muscle carotenoid concentration tended to be higher (10.0 and 8.6 mg kg⁻¹, respectively). Regardless of diet, the relative concentrations of astaxanthin *Z*-isomers in muscle and plasma were ca. 5 and 7%, respectively. However, the relative amount of 13*Z*-

astaxanthin was elevated pronouncedly in the liver (39-49% of total astaxanthin). Similarly, in a trial with astaxanthin (the astaxanthin source consisted of 75% all-*E*-, 3% 9*Z*- and 22% 13*Z*-astaxanthin) in a diet for Atlantic salmon, low levels of astaxanthin *Z*-isomers were present in plasma and muscle, whereas elevated levels of 13*Z*-astaxanthin were found in the liver (20-32% of total astaxanthin), Bjerkeng and Berge (2000). In Arctic charr (*Salvelinus alpinus*) fed a diet with *Z*-astaxanthins comprising 25% of total astaxanthin, low levels of *Z*-astaxanthins (ca. 5% of total astaxanthin) were detected in muscle (Bjerkeng *et al.* 2000a).

The most promising alternatives to synthesized astaxanthin for salmonid pigmentation today are apparently the red yeast *Phaffia rhodozyma* (Tangerås and Slinde, 1994) and *Haematococcus* alga (Lorenz and Cysewski, 2000). Recent studies on red yeast (Storebakken, Sørensen, Bjerkeng, Hiu, unpublished) and *Haematococcus* (Barbosa *et al.* 1999) indicate that properly disrupted cells have similar bioavailability as commercially formulated astaxanthin. Both oil-extracted pigment from langostilla (*Pleuroncodes planipes*) (Coral *et al.* 1998), red yeast (Choubert *et al.* 1995; Coral *et al.* 1998), and the microalga *Chlorella vulgaris* (Gouveia *et al.* 1996) were less efficient than Carophyll Pink (Hoffmann-La Roche, Basel, Switzerland) for muscle pigmentation of rainbow trout. Thus emphasis should be put on increasing bioavailability of microbial carotenoid sources. Experiments with red pepper (a non-astaxanthin source) have shown that this is a less efficient carotenoid source than commercially available astaxanthin sources (Carter *et al.* 1994; Yanar *et al.* 1997). Also oleoresin paprika pigments were less efficient than canthaxanthin in pigmentation of rainbow trout, and gave a less desirable color (Akhtar *et al.* 1999).

Dietary dose and dietary interactions

Dose-response relationships are in principle valid only for a specific dose formulation, under specific experimental conditions, for a specific species of a specific physiological condition and size. Linear blood response to increasing dietary dose of carotenoids indicate that uptake is governed by passive diffusion (Choubert *et al.* 1994; Kiessling *et al.* 1995; Storebakken and Goswami, 1996). Among the salmonid fish species, astaxanthin is least efficiently utilized in Atlantic salmon (cf. March and MacMillan, 1996). As with rainbow trout (reviewed by Storebakken and No, 1992), little extra flesh pigmentation is gained by using doses higher than ca. 50-60 mg/kg in diets for Atlantic salmon (Torrissen *et al.* 1995). However, due to low growth and drug treatments towards furunculosis, the numeric values of dose-response relationships should be used cautiously. Several factors may contribute to the particular dose-response relationships in salmon. Among these are reduced digestibility with increasing dose, metabolic losses, limited transport capacity, and limited uptake at the muscle cellular level.

Among the dietary macronutrients, lipids appear to affect pigmentation appreciably. No effects on pigmentation by proteins and carbohydrates have been reported. Recent reports confirmed higher astaxanthin deposition and retention in rainbow trout (Jensen *et al.* 1998) and Atlantic salmon (Bjerkeng *et al.* 1997b; Einen and Roem, 1997), by increasing dietary lipids. Nickell and Bromage (1998b) reported that L^* was significantly correlated to flesh lipid content and astaxanthin deposition was positively correlated to dietary lipid level. A higher dietary fat content increase astaxanthin deposition and CIE [1976] a^* -values in Atlantic salmon muscle at inclusion levels as high as 31 and 39%, respectively (Bjerkeng *et al.* 1997b). Sheehan *et al.* (1996), however, did not find any significant

influence of dietary fat content (21, 25 and 30%) on CIE [1976] a^* -values, whereas the a^* -values increased significantly with dietary fat content when measured directly on the surface of smoked fillets.

Fatter fish contain more astaxanthin (Bjerkeng *et al.* 1997b). However, the higher astaxanthin deposition in this study was mediated through increased absorption due to a higher dietary lipid level. The positive influence of dietary lipid levels on pigmentation and the lack of correlation between muscle lipid content and pigmentation are apparently contradictory, and merit closer investigation.

Oil fatty acid composition may influence deposition of carotenoids in fillets of Atlantic salmon. Thus, Atlantic salmon fed diets with high Peruvian high PUFA oil deposited about 13 % more carotenoids than fish fed diets supplemented with herring oil (Bjerkeng *et al.* 1999a; Bjerkeng *et al.* 2000b). An effect of similar magnitude was achieved by adding up to 800 mg α -tocopheryl acetate to diets for Atlantic salmon (Bjerkeng *et al.* 1999b).

Starvation, restricted feeding, and processing

Muscle astaxanthin level is not affected by starvation for up to 85 days (Foss *et al.* 1984; Choubert, 1985). It is therefore likely that the tissue depletion of astaxanthin during starvation in Atlantic salmon proceeds at a rate similar to whole muscle degradation. Higher ration level was associated with increased astaxanthin deposition during 110 d before slaughter, principally reflecting the increased dietary supply of astaxanthin (Einen *et al.* 1999). However, sensory scores for color intensity decreased and whiteness increased with increasing feed ration.

Slaughter procedures that involves different temperatures, handling and stress for the salmon, might affect color. Electrostimulation, of Atlantic salmon fillets gave lower Roche Colour Card for Salmonids scores and less red and a more yellow muscle (Robb *et al.*, 2000). Stressful conditions may give poorer fillet color of Atlantic salmon during harvested (Robb *et al.*, 2000). However, Sigholt *et al.* (1997) found no significant effect of pre-slaughter stress on colour of cooked fish as assessed by a sensory panel.

Much research effort has focused on absorption, deposition and utilization of various carotenoids in salmonid fishes, whereas effects of storage and processing on their stability have received less attention. The magnitude of the losses or changes in quality parameters in general may be highly variable and depend on the specific experimental conditions and notably the degree of processing used in the papers cited. Frozen storage quality of fishes is affected by oxygen availability, illumination and the presence of antioxidants, storage temperature, and of course duration. In a frozen storage trial with fillets of rainbow trout, aluminum laminated packaging materials (air-tight) were shown to be superior to ordinary vacuum bags (low oxygen transmission rate, OTR) and plastic wrapping in preventing rancidity and astaxanthin loss due to the low OTR and non-transparency (Bjerkeng and Johnsen, 1995). The deteriorative effect of oxygen was found to be higher than that of ordinary day-light. Also carotenoids themselves may act as antioxidants in muscle food-systems (Mortensen and Skibsted, 2000), as recently shown for canthaxanthin in patties from rainbow trout (Clark *et al.* 1999).

In a storage trial (4 °C) with vacuum-packaged muscle of rainbow trout fed diets with astaxanthin or canthaxanthin, effects on carotenoids on microbiological, oxidative stability, and color and pigmentation parameters were reported (Gobantes *et al.* 1998b). The authors found considerable losses of 32% astaxanthin, and 49% canthaxanthin during the 15 days storage. Colorimetric measures were

not well correlated to carotenoid concentrations. The authors concluded that astaxanthin was more stable than canthaxanthin during chilled storage. In a storage trial with Atlantic salmon, fish fed 573 and 865 mg α -tocopheryl acetate/kg diet had significantly higher color scores after 6 and 12 days fresh storage at 4 °C than fish fed 184 mg α -tocopheryl acetate/kg (Scaife *et al.* 2000). Therefore, increased dietary levels of Vitamin E (α -tocopheryl acetate) may improve coloration during fresh storage of fillets, in addition to the advantage of enhancing the pigment deposition (Bjerkeng *et al.* 1999b).

Most information regarding the effects of freezing and frozen storage on color and pigment content in salmonid flesh have emerged from studies on rainbow trout, studies on Atlantic salmon being scarcer. No and Storebakken (1991) performed a frozen storage experiment with fillets (skin on) of rainbow trout fed diets with either canthaxanthin or astaxanthin in polyethylene vacuum bags at -20 and -80 °C, respectively, for up to 6 months. Carotenoid losses were less than 5%. High stability of these carotenoids was also found by Scott *et al.* (1994). Christophersen *et al.* (1992) investigated the effect of OTR of packaging materials and exposure to light with different spectral distribution on the oxidative degradation of carotenoids in steaks of rainbow trout during frozen storage. They found that degradation of astaxanthin was sensitive to the radiant flux density of UV light, and less sensitive to OTR. Ingemansson *et al.* (1993) did not find any effect of astaxanthin on lipid hydrolysis and oxidation during frozen storage of rainbow trout muscle, whereas relative carotenoid losses were considerable. It should be pointed out, however, that the treatment groups only had 1.5 and 2.2 mg astaxanthin/kg white muscle, respectively, before the storage experiment started. Similarly, in the frozen and chill storage experiment with frozen and smoked rainbow trout fed different levels of vitamin E and fat, astaxanthin was found to not affect lipid oxidation (Jensen *et al.* 1998). On average, astaxanthin decrease amounted to 14% after 1 year of frozen storage, and 25% after 1.5 years. In general, only small changes were found during chilled storage, color and color stability being unaffected by vitamin E levels.

A few papers on the impact frozen storage on coloration of Atlantic salmon are cited in the following. According to Andersen and Steinsholt (1992), sensory evaluated redness of salmon stored at -35°C were higher than when stored at -13°C. Sheehan *et al.* (1998) addressed pigment stability and quality in Atlantic salmon fed diets with astaxanthin or canthaxanthin. The effect of frozen storage on stability of astaxanthin and canthaxanthin in raw and smoked salmon (ca. 2.25 kg) from different farms was investigated. No significant change in visual color score or carotenoid content was observed after 12 weeks in fish fed diets with astaxanthin (loss 5-15%). Significant losses were observed during frozen storage of salmon fed diets containing canthaxanthin, 50-60% lost after 12 weeks. Smoking of this fish after 6 and 12 weeks frozen storage, respectively, resulted in losses of astaxanthin of 15 and 23%, respectively. Significant effects of frozen storage temperature (-10, -20, and -30°C) but not storage time were observed on salmon wrapped in polyethylene bags and aluminum foils for up to 34 weeks by Refsgaard *et al.* (1998b). Quality during frozen storage may be improved by dietary vitamin E (Sigurgisladottir *et al.* 1994). However, in a frozen storage trial with Atlantic salmon, fish fed 573 and 865 mg α -tocopheryl acetate/kg diet no significant effect of α -tocopheryl acetate were found on color and pigmentation (Scaife *et al.* 2000).

Examination of cold-smoked salmon products has shown that products may exhibit substantial variation in exterior characteristics, in particular coloration. A considerable part of the products offered by the salmon retail industry allegedly have a yellowish hue. Although a consumer preference test concluded that most consumers prefer smoked cold-salmon with a light pink colour (Gormley, 1992), i.e. having a less yellowish hue. However, the variation in preference was considerable.

Lipid content has an influence on color parameters of cold-smoked Atlantic salmon. According to Rørå *et al.* (1998), a^* , b^* , hue and chroma increased significantly with increasing fat content of cold-smoked salmon. Similar findings were reported by Einen and Skrede (1998) and sensory assessed hue also was dependent on both astaxanthin and fat content of the raw muscle, hue changing towards more red with increasing pigment content and towards more yellow with increasing fat content. Also in hot-smoked rainbow trout red flesh color was negatively correlated to dry matter (fat) content (Rasmussen *et al.*, 2000). Thus, it may be concluded that not only pigment content but also fat levels of salmon should be considered as criteria when selecting raw salmon for the production of salmon with given color characteristics.

The cold-smoking process appears to have little effect on the pigment concentration. According to a recent study by Sheehan *et al.* (1998), cold-smoking of fresh Atlantic salmon fillets pigmented with either astaxanthin or canthaxanthin resulted in slight (9-13%), and statistically non-significant reductions of pigment concentrations. Freezing before cold-smoking seems to increase the pigment loss somewhat, and more so in the astaxanthin pigmented fish. Increased carotenoid content after cold-smoke processing of salmon is probably related to the shrinkage and water loss observed during processing.

Metabolic aspects

There are large differences among salmonid fish species in the capability to absorb and deposit carotenoids in the muscle (March and MacMillan, 1996). Thus as high muscle levels as 60 mg astaxanthin/kg may be found in sockeye salmon (*Oncorhynchus nerka*; Turujman *et al.* 1997), and up to 13 mg astaxanthin in Atlantic salmon (Bjerkeng *et al.* 1996). The amount of dietary astaxanthin that is utilized for flesh pigmentation rarely exceeds 15 % in Atlantic salmon (Torrissen *et al.* 1989) and 18 % in rainbow trout (Storebakken and No, 1992). This may be explained by poor uptake from the intestinal tract (faecal losses normally account for 30-70% of the dietary astaxanthin) and by poor retention of the absorbed astaxanthin in the muscle. Recently we found that only 40% of the absorbed astaxanthin was retained in the muscle of rainbow trout (Bjerkeng *et al.* 1997a). This may be explained by extensive metabolic transformation. The reductive pathway of 4-oxocarotenoids in salmonid fishes was recently reviewed by Schiedt (1998). The major limitations to accumulation of carotenoids by fish muscle are partly unclear. The uptake of astaxanthin and canthaxanthin from the intestine is slow, 18-30 h (March *et al.* 1990; Choubert *et al.* 1994). The blood clearance rate of carotenoids is slow and limited by the low rate of elimination from the different blood lipoprotein fractions (Guillou *et al.* 1992) However, the transport capacity of blood proteins appear to be about 100-fold higher than levels observed after normal feeding (Chavez *et al.* 1998). Also binding capacity of muscle actomyosin appears to be higher than observed maximum carotenoid levels (cf. Henmi *et al.* 1989).

We compared the apparent astaxanthin digestibility coefficients (ADC) and carotenoid compositions of the muscle, liver, whole kidney and plasma were compared in Atlantic salmon and the white-fleshed Atlantic halibut (*Hippoglossus hippoglossus*), Bjerkeng and Berge (2000). The ADC of astaxanthin was significantly and 10-30 % higher in Atlantic halibut than in Atlantic salmon. No astaxanthin was present in the muscle of the halibut and only small amounts of carotenoids were detected in plasma, liver and kidneys. However, also halibut appeared to be able to transform astaxanthin reductively into idoxanthin. This study indicate that the ability to metabolically transform ingested carotenoids plays an important role in carotenoid accumulation among fish species and thus flesh coloration.

Gobantes *et al.* (1997) showed that canthaxanthin was more rapidly cleared from the plasma of rainbow trout than astaxanthin. This indicates that the metabolic transformation rate of canthaxanthin is higher than for astaxanthin. We investigated the blood appearance and transport of astaxanthin, and catabolic transformation of astaxanthin to idoxanthin in Atlantic salmon force-fed single doses of ^{14}C -astaxanthin (Aas *et al.* 1999; Fig. 5). Idoxanthin appeared rapidly in the blood and maximum carotenoid concentration was reached after 30 h (Fig. 6). Separation of plasma by ultracentrifugation showed that 58 % of the radiolabeled carotenoids were present in a high-density protein fraction not containing lipoproteins, presumably albumin, and 42% were present in lipoproteins.

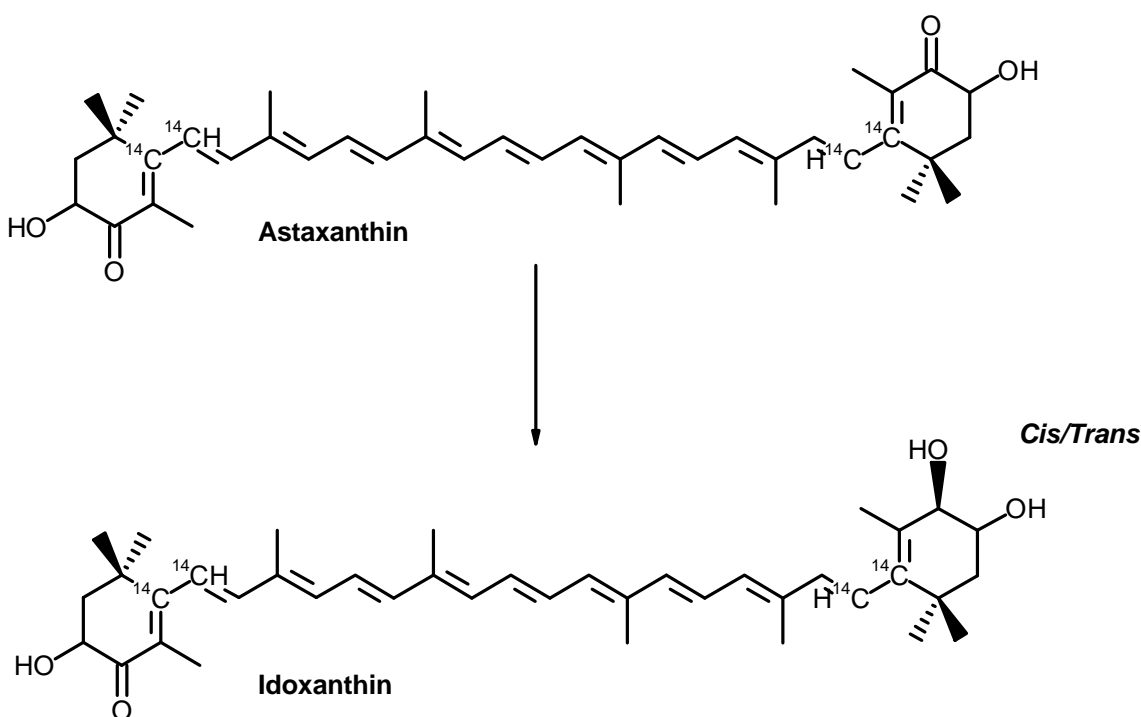


Figure 5. Conversion of $[6,7,6',7'-^{14}\text{C}_4]$ -astaxanthin to $[6,7,6',7'-^{14}\text{C}_4]$ -idoxanthin.

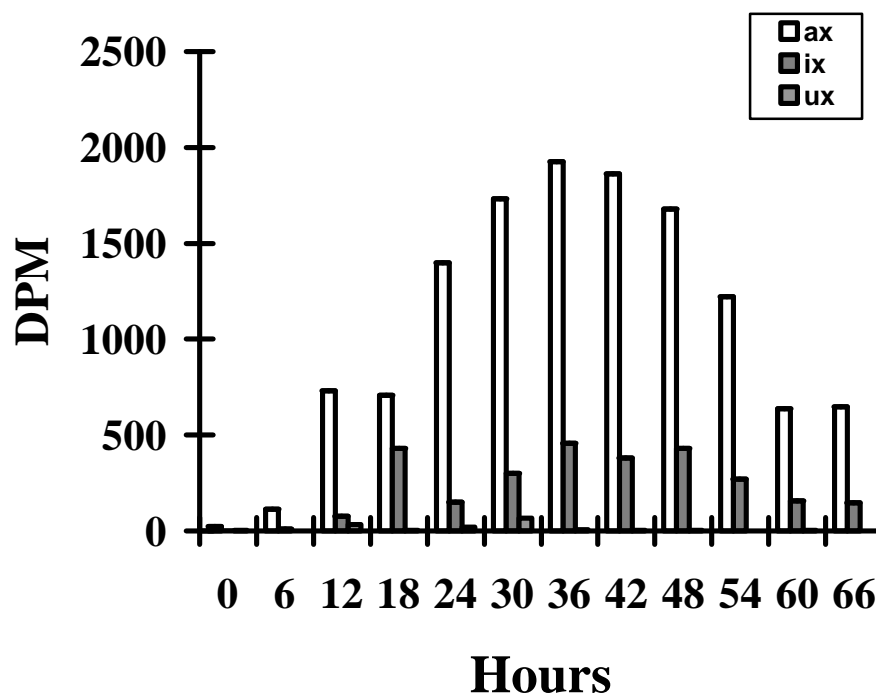


Figure 6. Radioactivity ($\text{dpm } \mu\text{l}^{-1}$) in total, ^{14}C -astaxanthin (AX), combined 3',4'-*cis* and 3',4'-*trans* glycolic isomers of ^{14}C -idoxanthin (IX), and unidentified radiolabeled compounds (UC) in three Atlantic salmon from 6 to 66 h after force-feeding $[6,7,6',7'-^{14}\text{C}_4]$ -astaxanthin.

Our work with Arctic charr has consistently shown that it contains rather large amounts of idoxanthin (46-76% of total carotenoids; Aas *et al.* 1997; Hatlen *et al.* 1997; Bjerkgeng *et al.* 1999c). Sexually maturing salmonid fishes (Steven, 1949), including Arctic charr (Hatlen *et al.* 1995, 1996), redistribute their body pool of carotenoid pigments, there being a transfer of flesh carotenoids to the skin and gonads. It should be noted that the extent of pigment alterations during sexual maturation vary considerably between salmonid fish species. Sexual maturation is also associated with a prominent loss of whole-body carotenoids (Bjerkgeng *et al.* 1992). Our investigations of effects of implants of 11-ketotestosterone (11KT), 17 β -estradiol (E_2), and 3,5,3'-triiodo-L-thyronine (T_3) showed that 11KT implantation reduced astaxanthin and idoxanthin concentrations of plasma and fillets, and increased the amount in liver and skin (Bjerkgeng *et al.*, 1999a). Male Arctic charr had significantly higher carotenoid content in plasma, fillet and skin than female fish. In rainbow trout plasma, however, levels of astaxanthin and canthaxanthin were higher in sexually mature females than males (Gobantes *et al.* 1998b). Thus sex hormones affect carotenoid metabolism and partitioning among body compartments, effects that are differently displayed by the sexes.

Variations in metabolic and muscle clearance rate may contribute to the overall variation in pigmentation and coloration. The relatively low retention of astaxanthin in Atlantic salmon may be related to a lower absorption, muscle affinity, or higher metabolic turnover of astaxanthin in this species. Further studies are needed to clarify the interrelationships between salmonid carotenoid

metabolic turnover and carotenoid deposition. More direct indicators of the rate of carotenoid turnover should be developed.

CONCLUDING REMARKS

Apart from imparting a desirable coloration, evidence for beneficial biological actions of carotenoids in salmonid fishes is emerging. Thus feeding diets supplemented with synthesized astaxanthin or red yeast reduce serum lipid peroxide content (Nakano *et al.* 1995), reduce susceptibility of liver to lipid oxidation (Nakano *et al.* 1999a), and normalize liver function and reduce serum lipids and lipid peroxides in rainbow trout fed oxidized oil (Nakano *et al.* 1999b). Reduced levels of plasma triacyl glycerols were observed by Rehulka (2000). Astaxanthin exhibited a negative effect on liver and muscle catalase activity (Lygren *et al.* 1999), whereas no effect of astaxanthin on phagocytic production of reactive oxygen species were observed in head kidney of Atlantic salmon (Lygren and Waagbø, 1999). Dietary deficiency of astaxanthin increased the hepatocytic recovery of desaturated and elongated products of polyunsaturated fatty acids (Bell *et al.* 2000). These effects may explain the positive effects of astaxanthin on growth and disease resistance on Atlantic salmon (Christiansen *et al.* 1995b).

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