

Pigmentation of Salmonids — Carotenoid Deposition and Metabolism*

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I. INTRODUCTION

The pink to red color of the flesh of anadromous salmonids (*Salmo* spp., *Oncorhynchus* spp., and *Salvelinus* spp.) is one of the distinguishing features of these fishes and makes a major contribution to their elite image. It is therefore of great economic importance that these fishes, either wild or farmed, are pigmented to meet consumer preferences. This pigmentation is due to absorption and deposition of oxygenated carotenoids.

Carotenoids are widespread and important pigments and are found in all families in the vegetable and animal kingdoms.^{1,2} Their bright color is due to a chromophore consisting of a chain of conjugated double bonds (Figure 1). Only plants and protists are able to synthesize carotenoids, so fish like other animals must obtain carotenoids from dietary sources.^{1,3}

Salmonid aquaculture production has increased greatly in the past decade and production levels are expected to continue to rise. The use of carotenoid pigments in the feeds of farmed salmon has also increased. In 1986, for example, over 6000 kg of carotenoid pigments were used in the diets of farmed salmonids. At a cost of over \$1000 U.S./kg for synthesized products, the investment in carotenoids was substantial. Salmon feed costs were increased approximately 10 to 15% by carotenoid supplementation. By 1990, nearly 15,000 kg of carotenoid pigments will be used in salmon feeds to meet the predicted needs of the industry. Despite the relatively high costs of carotenoids and their poor retention in the flesh, little research has been done to elucidate the factors affecting absorption and deposition or the metabolic turnover and biological functions of various carotenoid pigments in salmonids.⁴⁻⁶

In this paper, we review pigmentation of salmonids with special emphasis on alternative pigment sources, factors influencing absorption and deposition, metabolism, and biological functions. Proposed functions of carotenoids in the reproductive cycle have recently been reviewed and are not covered here.^{9,10}

II. GENERAL REMARKS

Variation among laboratories in analytical methods used to quantify and identify carotenoids and incomplete reporting of

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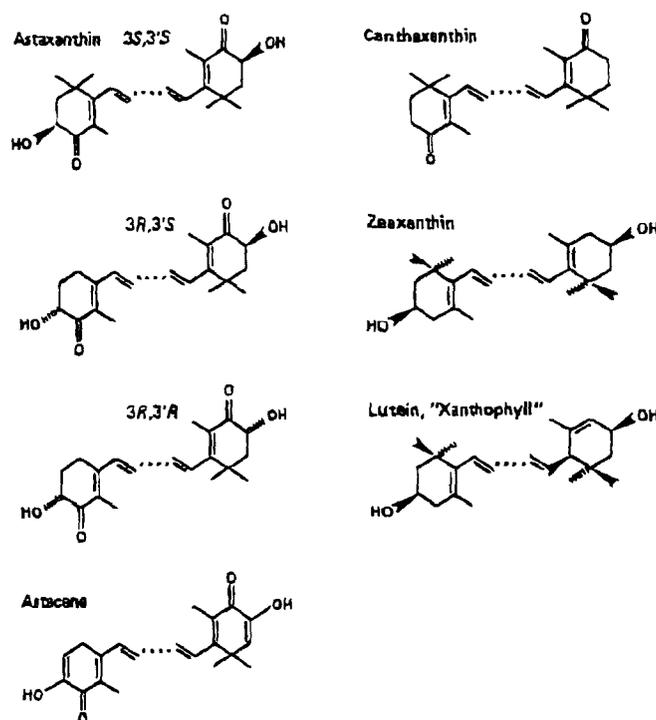


FIGURE 1. Structure of selected carotenoids. Also see Figure 3. (Based on data from References 3 and 4.)

critical aspects of the experimental design or failure to properly design carotenoid studies make it difficult to obtain a comprehensive picture of the current state of knowledge of carotenoid deposition and metabolism in salmonids.

Analytical work with carotenoids has been hampered by the limited availability of standards, and standards of important salmonid carotenoids such as astaxanthin and canthaxanthin are not commercially available. Carotenoids occur naturally in small amounts as mixtures of related compounds. They are labile, and oxygenation, isomerization, and rearrangement occur easily. Purification of individual carotenoids from natural

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sources for use as analytical standards has been difficult. Therefore, many authors have attempted to quantify carotenoids in samples using procedures of questionable accuracy.

For example, methods reported in the literature for quantification and identification of carotenoids often seem designed to fit individual preferences or the equipment available without regard to standardization among laboratories. This is clearly shown by the large range in extinction coefficients used in quantification of astaxanthin in acetone: $E_{1\text{cm}}^{1\%} = 1600$;¹¹ $E_{1\text{cm}}^{1\%} = 2100$;¹² and $E_{1\text{cm}}^{1\%} = 2200$.¹³ Due to a lack of standards, these extinction coefficients are often calculated from other more easily obtained carotenoids (β -carotene)¹⁴ and extrapolations are made to fit different solvents or expected ratios of stereoisomers. This makes it difficult to compare absolute values of carotenoid levels in fish tissue or feeds reported by various authors.

In previous work, astacene (3,3'-dihydroxy-4,4'-diketo-2,3,2',3'-tetrahydro- β -carotene; Figure 1) was reported as the red pigment of salmonids.^{5,15} Later work showed that astacene was an artifact of astaxanthin, therefore astacene is hereafter referred to as astaxanthin in this review.

Differences in experimental conditions and treatment of samples between experiments conducted by various workers have complicated comparisons of results and, in some cases, compromised the value of their results. In many experiments, samples to be examined for carotenoid concentration have been stored frozen for a considerable time. Carotenoids degenerate rapidly, even in frozen samples, so some of the values reported in the literature may be in error.¹⁶ Information on initial and final fish weight, growth rate, feed composition, and feed consumption must be reported in scientific papers to permit critical evaluation of work in this field. Very few papers in the salmonid carotenoid area report complete information on experimental design and conditions, thus making it impossible to compare the results of different authors. Use of the International Council for the Exploration of the Seas (ICES) recommendations on experimental design, diet formulation, and methods for measurements and evaluation would improve greatly the value of future work.¹⁷ Improved analytical methods, primarily high-performance liquid chromatography (HPLC), have recently made it possible to isolate and quantify minute quantities of carotenoids and their stereoisomers.^{4,18} This will expand the possibilities for progress in this field.

III. PRIMARY SALMONID CAROTENOIDS

Astaxanthin (3,3'-dihydroxy-4,4'-diketo- β -carotene; Figure 1) is the main carotenoid pigment of red/pink colored aquatic animals.¹⁹⁻²² In wild salmonids, astaxanthin and its esters are obtained from ingestion of either zooplankton or fish that have zooplankton in their digestive tract. About 90% of the carotenoids found in the tissue are located in the flesh in their free form, but large amounts are also found in the skin and ovaries

in maturing fish.^{5,23} Hydroxy-carotenoids in the skin are present mainly as esters.²⁴

Steven^{5,15} showed that astaxanthin is deposited in the chromatophores in the skin of brown trout (*Salmo trutta*). The site of carotenoid deposition in salmonid flesh is not known. Intermuscular fat in salmonids has a pale color without visible carotenoid content. This suggests that specific carotenoid-binding proteins or lipoproteins are present in the muscle. Burton and Ingold²⁵ predict that carotenoids will tend to concentrate in membranes and organelles exposed to the lowest partial pressure of oxygen. In salmonid eggs, the carotenoid is bound to proteins, probably lipovitellin.²⁶

The reported carotenoid levels of wild salmonids are shown in Table 1. The levels of carotenoids in farmed salmonids show large variations among individuals of the same species, and the values reported for wild salmonids may represent more than comparative differences in the ability to deposit carotenoids.^{30,31} Factors which also may contribute to observed differences are dietary pigment source, fish size, stage of maturation, and genetic differences.

Carotenoids are responsible for the red to pink color of salmonid flesh, but the relationship between visual score and carotenoid level is linear only at low carotenoid levels in farmed fish (Figure 2). At least two factors contribute to this: the human eye seems to be less sensitive to carotenoid concentrations over 3 to 4 mg/kg compared with lower concentrations, and unpigmented intermuscular fat may mask the impression of color.

Based on visual color impression, a level of 3 to 4 mg/kg can be regarded as an acceptable carotenoid concentration in marketable farmed salmon. Carotenoids may fade in salmonid flesh during storage and processing, and compensation must be made for this by elevating flesh carotenoids slightly above 4 mg/kg to ensure acceptable levels in the delivered product.

A. Specific Carotenoids

Salmonids are not able to oxygenate carotenoids, but deposit ingested oxygenated carotenoids without modification.^{3,35} The farmed salmonids show a more complex carotenoid picture than wild salmonids because a wider range of feed ingredients containing a variety of pigments are used.³⁶ Two oxygenated,

Table 1
Carotenoid Levels Reported in Wild Salmonids

Species	Carotenoids (mg/kg)	Ref.
Sockeye salmon (<i>Oncorhynchus nerka</i>)	26-37	20, 27
Coho salmon (<i>O. kisutch</i>)	9-21	20, 27
Chum salmon (<i>O. keta</i>)	3-8	20, 27
Chinook salmon (<i>O. tshawytscha</i>)	8-9	20
Pink salmon (<i>O. gorbuscha</i>)	4-6	20
Atlantic salmon (<i>Salmo salar</i>)	3-11	13, 27, 28
Rainbow trout (<i>S. gairdneri</i>)	1-3	29

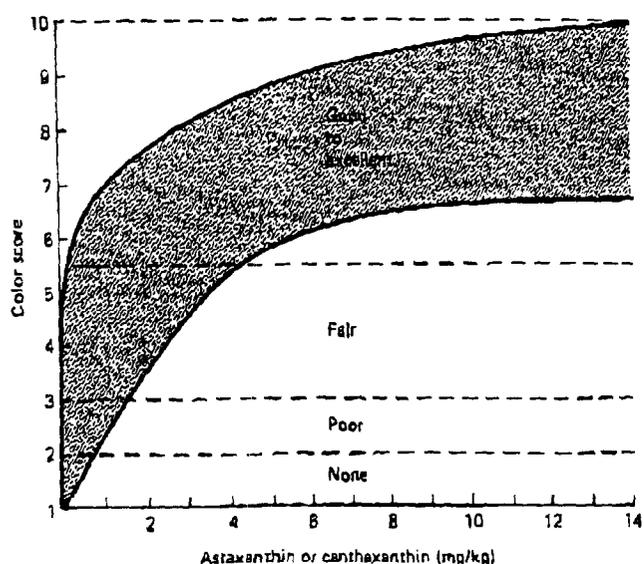


FIGURE 2. The relationship between visual coloration score and carotenoid concentration in salmonid flesh. (Based on data from References 16 and 32 to 34.)

red carotenoids are readily deposited in salmonid flesh: **astaxanthin and canthaxanthin** (4-4'-diketo- β -carotene; Figure 1).^{5,24,37-41} Several other carotenoids have also been isolated in minor amounts in both farmed and wild salmonids (Table 2).

In goldfish, differences in the rates of accumulation of carotenoids are due to differences in absorption, and it has

been suggested that absorption is enhanced by incorporation of hydroxyl groups into the carotene skeleton.⁴⁴ This hypothesis is supported in salmonids by various authors, who have shown that astaxanthin is deposited at significantly higher levels than canthaxanthin in both Atlantic salmon (*S. salar*) and rainbow trout (*S. gairdneri*).^{38,45-47} Schiedt et al.⁴³ found preferred deposition of astaxanthin followed by adonirubin (3-hydroxy-4,4'-diketo- β -carotene) and canthaxanthin, while zeaxanthin ((3R,3'R)- β , β -carotene-3,3'-diol; Figure 1) and lutein (3,3'-dihydroxy- α -carotene; Figure 1) were not absorbed as well, and β -carotene (Figure 3) was poorly absorbed. This fits the model of Hata and Hata,⁴⁴ with the exception of zeaxanthin and lutein which, on the basis of hydroxyl groups, should be absorbed at levels between astaxanthin and adonirubin.

The absorption efficiency of various carotenoid pigments differs among animal groups. Mammals absorb β -carotene, while fish and birds prefer oxygenated carotenoids. Salmonids absorb astaxanthin and canthaxanthin 10 to 20 times more efficiently than they absorb lutein and zeaxanthin, while chickens absorb zeaxanthin at 3 times the rate of astaxanthin.⁴³ Goldfish and fancy red carp are similar to the chicken in their absorption preference: astaxanthin \rightarrow zeaxanthin \rightarrow lutein.^{44,48} Thus, salmonids preferentially absorb 4-4'-keto-carotenoids while goldfish and fancy red carp absorb the 3-3'-hydroxy-carotenoids.

B. Astaxanthin Esters

Dietary astaxanthin esters seem to be absorbed by salmonids to a lesser degree than free astaxanthin. This was demonstrated

Table 2
Relative Distribution of Carotenoids in Salmonids

Carotenoid	Muscle	Skin	Ovaries	Liver	Kidney	Serum
3-Epilutein	(X)					
3-Epilutein-ester		(X)				
Astaxanthin-diester		XXX				
Astaxanthin-monoester		X			X	
Astaxanthin-free	XXXXX*	(X)*	XXXXX*	(X)*	X*	XXXXX*
Canthaxanthin	XXXXX*	XXX*	XXXXX*			XXXXX*
Cyanoaxanthin	(X)		(X)			
Diatroxanthin	(X)		(X)			
Doradexanthin	(X)		(X)			
Lutein					XX	
Lutein-esters		XXX			XX	
Zeaxanthin	X		X		X	
Zeaxanthin-esters		XX			X	
Zeaxanthin-5,6-epoxide		X				
Cryptoxanthin		(X)				
Echinonone		(X)				
β -Carotene		(X)				
Deepoxyneoxanthin		(X)				
Adonirubin		(X)				
Asteroidenone		(X)				

Note: Based on data from References 23, 27, 29, 42, and 43.

* Depending on dietary carotenoid source.

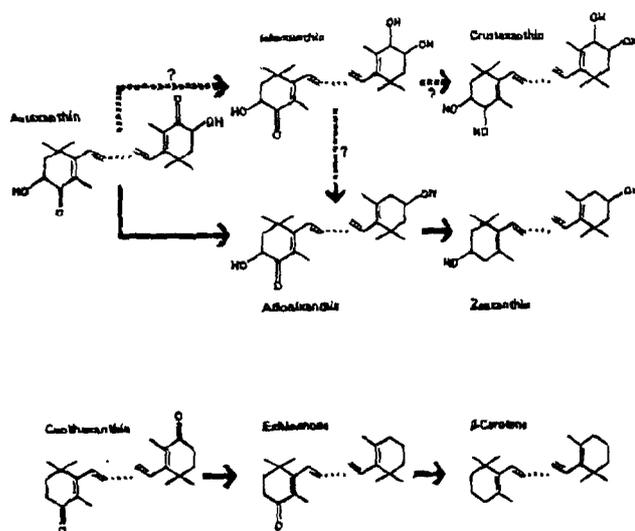


FIGURE 3. Reductive metabolism of astaxanthin and canthaxanthin in salmonids. (Based on data from References 23, 43, and 56.)

for astaxanthin diesters and monoesters and free astaxanthin purified from the copepod, *Calanus finmarchicus*.⁷ A similar low deposition of astaxanthin in the flesh from astaxanthin dipalmitate was observed by comparing synthetic astaxanthin dipalmitate and free astaxanthin as pigment sources for rainbow trout, sea trout (*S. trutta*), and Atlantic salmon.^{32,33} Only free astaxanthin is found in the flesh and plasma, indicating that astaxanthin esters are hydrolyzed in the digestive tract and that astaxanthin is absorbed in the free state.^{5,21,24,29,42,49} The rate of hydrolysis of astaxanthin esters to free astaxanthin appears to be limited, and this might explain the observed differences in deposition.

In crustacea, a relatively large part of the astaxanthin is in the esterified form; yet, in most feeding experiments, crustacea and crustacean waste products seem to be more efficient pigment sources than would be expected based on their levels of free astaxanthin.^{7,14,50-53} The reason for this unexpected observation is unknown, but may be related to other compounds in crustacea which facilitate absorption and deposition of carotenoid pigments.

C. Isomers

In marine fish, astaxanthin occurs as a mixture of three optical isomers: (3R-3'R), (3R-3'S), and (3S-3'S) (Figure 1).⁴ The ratio of the astaxanthin isomers deposited in the flesh of Atlantic salmon, rainbow trout, and sea trout reflects the chirality of the ingested astaxanthin, but epimerization from the 3S to the 3R form seems to occur during the reductive metabolism of astaxanthin to zeaxanthin.^{32,33,45,54,55} Ando³⁶ noticed the same ratio among the three optical isomers in ovaries as in the flesh of chum salmon (*Oncorhynchus keta*), indicating that there is no preferential transport or deposition in eggs of the optical isomers of astaxanthin.

Higher ratios of (3R-3'R) astaxanthin were found in the flesh of rainbow trout and sea trout fed racemic astaxanthin (1 [3R-3'R]: 2 [3R-3'S]: 1[3S-3'S]) dipalmitate than the (3S-3'S) form, indicating that the esterases hydrolyze astaxanthin (3R-3'R) dipalmitate more efficiently than astaxanthin (3S-3'S) dipalmitate, resulting in higher rates of deposition in the flesh of the (3R-3'R) astaxanthin.³² This corresponds with the results of Katsuyama et al.,⁵⁵ who fed rainbow trout purified stereoisomers of astaxanthin diester from *Euphausia superba*, *Palaeomon pacificus*, and *P. paucidens* and observed a two times higher deposition of (3R-3'R) astaxanthin than (3R-3'S) astaxanthin and a four times higher deposition of (3R-3'R) astaxanthin than (3S-3'S) astaxanthin in the flesh.

IV. CAROTENOID SOURCES

A. Crustacea and Crustacean Byproducts

Astaxanthin is the major carotenoid in many crustaceans and is present in free and esterified forms and as protein complexes.^{1,7,11,56} Great effort has been made to evaluate different crustacean products as carotenoid sources for farmed fish. Table 3 shows the carotenoid level in several products and Table 4 shows a summary of the results achieved in feeding experiments. The main factor determining the value of these pigment sources seems to be their astaxanthin concentration. The level of carotenoid in most crustaceans is relatively low. To achieve satisfactory pigmentation of salmonids, the diet must contain 10 to 25% of this material. Crustacean wastes contain low levels of protein and high levels of moisture, ash, and chitin, which limits the percentage of these products that can be included in salmonid feeds.^{34,76}

In Norway, waste from shelling shrimp (*Pandalus borealis*) is the traditional, natural pigment source for Atlantic salmon and rainbow trout. The astaxanthin level in hand-shelled shrimp waste is usually higher than in waste shelled by machine (Table 3). This is due mainly to the tendering of the raw shrimp before shelling, but is also due to extensive washing of the machine-shelled product. Shrimp for hand shelling are boiled immediately after being caught, while machine-shelled shrimp are stored on ice for 6 to 7 d before shelling. Ugleiveit⁵¹ and Torrissen⁷⁹ have shown that shrimp waste can produce an acceptable pigmentation of rainbow trout when included as 10 to 20% of the diet for about 7 months (Table 4).

Krill (*E. superba*, *E. pacifica*), the copepod *C. finmarchicus*, and the red crab (*Pleuroncodes planipes*) have been shown to pigment the flesh of salmonids (Table 4).^{7,50,75,77} Recently, methods for extraction of carotenoprotein from shrimp (*Pandalus borealis*) waste have been developed that have the potential for providing carotenoids as feed supplements in rations of farmed fish.^{64,78} Their use in commercial production of salmonids will depend upon the production cost and the level and availability of the astaxanthin in these products.

Dry-pelleted diets are increasingly used to rear salmonids,

Table 3
Carotenoid Content in Some Pigment Sources Used for Pigmenting Salmonids

Pigment source/treatment	Carotenoid	Amount (mg/kg)*	Ref.
Crustacean			
Krill (<i>Euphausia</i> spp.)	Astaxanthin	22-77	14, 37
	Free	(3-31%)	
	Monoester	(36-49%)	
	Diester	(33-48%)	
Krill (<i>E. pacifica</i>)	Astaxanthin	100-130	52
Krill (<i>Meganyctiphanes norvegica</i>)	Astaxanthin	46-83	57
Copepode (<i>Calanus finmarchicus</i>)	Astaxanthin	39-84	14, 58
	Free	(25%)	
	Monoester	(38%)	
	Diester	(37%)	
Red crab (<i>Pleuroncodes planipes</i>)	Astaxanthin	100-160	30, 52
Crustacean waste			
Shrimp (<i>Pandalus borealis</i>), hand shelled	Astaxanthin	60-128	59
Shrimp (<i>P. borealis</i>), machine shelled	Astaxanthin	20-48	60
Shrimp (<i>P. borealis</i>), slaged	Astaxanthin	74	53
Crustacean meals			
Crab (<i>Chinooketes opilio</i>), vacuum dried	"Lutein-like"	0.1	41
	Astaxanthin	5	
	Astacene	2	
	Astaxanthin	76	62
Crab (<i>Greyon quinquecostis</i>), freeze-dried	Astaxanthin	200	62
Krill (<i>Euphausia</i> spp.), co-dried with oil	Astaxanthin	137	79
Crawfish meal (<i>Procambarus clarkii</i>)	Free	(45%)	
	Esters	(55%)	
	Astacene	16	
	Astaxanthin	100	41
Shrimp (<i>Pandalus borealis</i>), vacuum dried	Unidentified	2	
	Astacene	9	
	Astaxanthin	192	53
Shrimp (<i>P. borealis</i>), steam dried + antioxidant			
Crustacean extract			
Red crab (<i>Pleuroncodes planipes</i>), oil extract	Astaxanthin	1,550	34
Crawfish (<i>Procambarus clarkii</i>), oil extract	Astaxanthin	750	63
Copepode (<i>Calanus finmarchicus</i>), oil	Astaxanthin	520	14
	Free	(40%)	
	Monoester	(33%)	
	Diester	(27%)	
	Astaxanthin	1,160	64
Shrimp (<i>P. borealis</i>), freeze-dried carotenoprotein			

Table 3 (continued)
Carotenoid Content in Some Pigment Sources Used for Pigmenting Salmonids

Pigment source/treatment	Carotenoid	Amount (mg/kg)*	Ref.
Fish oils			
Capelin (<i>Mallotus villosus</i>) oil	Astaxanthin	6-94	14
Mackerel	Astaxanthin	6-11	
Krill oil (<i>Euphausiids</i> spp.)	Astaxanthin	727	14
	Free	(6%)	
	Monoester	(43%)	
	Diester	(51%)	
Shrimp oil (<i>Pandalus borealis</i>)	Astaxanthin	1,095	14
	Free	(3%)	
	Monoester	(18%)	
	Diester	(79%)	
Plant and plant products			
Marigold flowers (<i>Tagetes erecta</i>), extract	Lutein	(90%)	65
Squash flowers (<i>Cucurbita maria</i>), extract	Zeaxanthin	(38%)	65
	Lutein	(23%)	
	β -Cryptoxanthin	(17%)	
	(Capsanthin, capsorubin)	235	2, 66
Paprika extract			
<i>Hyppophae rhamnoides</i> oil	α -Carotene	4	67
	β -Carotene	298	
	γ -Carotene	49	
	Cryptoxanthin	80	
	Lycopene	28	
	Lutein	20	
	Zeaxanthin	41	
	Violaxanthin	19	
Algae			
<i>Spirulina</i> spp., spray dried	β -Carotene	434	68
	β -Carotene-5,6-epoxide	79	
	Echinonope	118	
	Cryptoxanthin	389	
	Zeaxanthin	151	
	Myxoxanthophylli	409	
	O. glycomides	107	
Yeast			
<i>Rhodospirula sarnell</i>	β -Carotene	7	69
	γ -Carotene	1	
	Torularhodin	83	
	Torulene	28	
<i>Phaffia rhodosyne</i>	Astaxanthin	30-800	11, 73
Synthetic products			
"Carophyll red" ^{††} / ^{†††} Roxanthin red ^{††}	Canthaxanthin	100,000	
"Carophyll pink" ^{††}	Astaxanthin	50,000	

* Values in bracket give percent distribution of astaxanthin forms (free astaxanthin, astaxanthin monoester, and astaxanthin diester) or distribution of different carotenoids.
[†] Based on carotenoid declared in diet.
^{††} Hoffman La Roche, Basel, Switzerland.

Table 4
Pigment Deposition In Atlantic Salmon, Coho Salmon, Brook Trout, Rainbow Trout, and Sea Trout
by Different Pigment Sources and Diet Levels of Carotenoids

Pigment sources	Carotenoid ^a	Conc (mg/kg) ^b	Days of feeding	Initial weight (g)	Final weight (g)	Conc (mg/kg) ^c	Retention (%)	Ref.
<i>Atlantic salmon (Salmo salar)</i>								
Shrimp waste	A	(7) 13	520	500	2700	4.9		46
Shrimp-waste silage	A	(6) 13	520	500	2600	5.2		46
Synthetic	A	30-90	392	62	400	2.0		33
	A	30	392	62	400	0.9		33
	A	60	392	62	400	2.3		33
	A	90	392	62	400	2.6		33
	A-RM	100	105	1230	1570	0.5		54
	A-RR	100	105	1230	1570	0.7		54
	A-RS	100	105	1230	1570	0.3		54
	A-SS	100	105	1230	1570	0.4		54
	ADP	30-90	392	62	400	0.7		33
	C	43	430	53	1200	6.1		70
	C	46	430	53	1400	4.1		70
	C	100	105	1230	1570	0.8		54
	C	(9)18	520	500	2200	6.3		46
	C	30-90	392	62	400	1.8		33
<i>Coho salmon (Oncorhynchus kisutch)</i>								
Crayfish waste	A	50	133	82	226	3.5		71
<i>Pleuroncodes planipes</i> extract	A	(30) 48	155	80	205	2.3		34
<i>P. planipes</i> extract	A	(60) 97	155	80	205	2.6		34
<i>P. planipes</i> extract	A	(90) 145	155	80	205	2.7		34
Synthetic	A	50	133	82	222	6.4		71
	A + C (1:1)	50	133	82	217	7.3		71
	C	50	133	82	220	5.4		71
	C	75	133	82	208	6.1		71
	C	100	133	82	206	6.5		71
<i>Brook trout (Salvelinus fontinalis)</i>								
<i>Chinchoetes optila</i>	A	(1)	84	49	92	(1.8) ^d		41
Paprika	M	(49)	49		80	3.2		66
Shrimp waste, vacuum dried	A	(20)	84	49	82	(14.3)		41
	A	(30)	56	49	68	(4.8)		41
Synthetic	C	(40)	56	49	67	(19.8)		41
<i>Rainbow trout (Salmo gairdneri)</i>								
<i>Calanus finmarchicus</i>	A	(11.6) 20	33	250		0.7	12.7	7
<i>C. finmarchicus</i> , extract	A	(12.7) 22	33	250		0.8		7
	AD	(13.0) 22	33	250		0.3	4.5	7
	AD	(25.9) 45	33	250		0.5		7
	AD	(60.7) 105	33	250		0.6	2.2	7
	AF	(9.3) 16	33	250		0.8	18.0	7
	AM	(12.5) 21	33	250		0.6	10.3	7
<i>Cucurbita maxima</i> , flowers	M	100	56	127	152	0.0		65
<i>Euphausiid</i> /shrimp waste ^e	A	28	75	120	232	5.0		52
<i>Euphausiid</i> /shrimp waste ^f	A	28	75	120	230	4.2		52
<i>Greyon quinquegens</i> , extract	A	100	28	132	169	0.9		62
<i>Greyon quinquegens</i>	A	15	28	132	165	0.1		62
<i>Hypophae rhamnoides</i> oil	M	46	28	130	173	0.2		67
Krill meal	A	(1.5) 2.5	120	90		1.4	60	72
	A	(3.0) 5.0	120	90		1.5	30	72
	A	(4.5) 7.5	120	90		1.8	20	72
	A	(4.5) 7.5	250	250		1.5		72
<i>Phaffia rhodosyna</i> , broken	A	(55) 82	48	70		9.2		73
	A	(55) 82	42	70		6.5		73

Table 4 (continued)

Pigment Deposition in Atlantic Salmon, Coho Salmon, Brook Trout, Rainbow Trout, and Sea Trout by Different Pigment Sources and Diet Levels of Carotenoids

Pigment sources	Carotenoid ^a	Conc (mg/kg) ^b	Days of feeding	Initial weight (g)	Final weight (g)	Conc (mg/kg) ^c	Retention (%)	Ref.
<i>P. rhodozyma</i> , digested	A	(55) 82	42	70		11.9		73
<i>P. rhodozyma</i> , intact	A	(55) 82	87	70		0.6		73
<i>P. rhodozyma</i> , oil extract	A	(80) 119	42	70		5.1		73
<i>P. rhodozyma</i> , partly digested	A	(55) 82	42	70		2.5		73
<i>P. rhodozyma</i> , whole	A	(60) 90	42	70		1.5		73
<i>Pleuroncodes planipes</i> ^d	A	(8.7) 14	63	108	328	1.4		74
<i>P. planipes</i> ^e	A	(8.7) 14	63	107	317	1.6		74
<i>Rhodortula sanneii</i>	M	1199			180	3.6		69
Shrimp meal	A	(4.8) 14	87	480	681	1.4		53
Shrimp meal ^f	A	10		100	190	(0.9)		8
Shrimp meal ^g	A	11		100	190	(1.4)		8
Shrimp meal ^h	A	12		100	190	(1.3)		8
	A	(3.4) 10	225	28	225	1.2		75
	A	(4.9) 15	87	480	662	1.5		53
	A	(6.0) 19	225	28	225	1.3		75
	A	(6.1) 14	105	135		1.1		46
	A	(12.1) 38	225	28	225	1.8		75
Shrimp-waste silage	A	(4.9) 15	87	480	657	2.0		53
<i>Spirulina</i> spp.	M	0-339	56	100	200	0.0		68
Synthetic	A	(4.9) 12	105	135		1.4		46
	A + C(1:1)	60	154	93	400	10.7		32
	A + C(36:64)	75	65	275	350	4.5		38
	A-RM	71	98	350	540	5.7		45
	A-RR	83	98	350	540	7.2		45
	A-RS	74	98	350	540	8.2		45
	A-SS	88	98	350	540	5.7		45
	ADP + C	60	154	93	400	10.4		32
	C	(5.8) 14	105	135		1.4		46
	C	43	430	176	2900	13.7		70
	C	46	430	176	3100	10.2		70
	C	(800)		262		18.3		69
	C	111	98	350	540	2.4		45
	C	120	87	70		7.5		73
<i>Tagetes erecta</i> flowers ⁱ	M	(100)	32	127	164	0.01		65
Sea Trout (<i>Salmo trutta</i>)	A + C(1:1)	60	154	48	86	1.8		32
Synthetic	ADP + C(1:1)	60	154	48	86	1.9		32
	C	43	430	78	400	12.4		70
	C	46	430	78	350	4.9		70

^a A = astaxanthin, C = canthaxanthin, M = multiple (see Table 3), A-RR = (3R-3'R) astaxanthin, A-SS = (3S-3'S) astaxanthin, A-RM = racemic astaxanthin, AF = free astaxanthin, AM = astaxanthin monoester, AD = astaxanthin diester, A + C = astaxanthin + canthaxanthin, ADP + C = astaxanthin dipalmitate + canthaxanthin.

^b Values in parenthesis = mg/kg wet diet.

^c Values in parenthesis = mg/kg dry muscle.

^d Mainly lutein and astaxene.

^e 15% fat in the diet.

^f 10% fat in the diet.

^g 9.5% fat in the diet.

^h 12.3% fat in the diet.

ⁱ 13% fat in the diet.

^j 9% fat in the diet.

^k 17% fat in the diet.

and high moisture products cannot be used as constituents in dry diets. Dry meals and oil extracts containing carotenoids are being examined as dietary pigment sources in salmonid diets to increase the possible utilization of natural pigment sources and to reduce handling costs.^{34,61,79,80}

The level of astaxanthin in shrimp meal varies from 0 to about 200 mg/kg, depending on the quality of the shrimp waste, the species, the drying method used, and whether antioxidants are added to the product.^{14,52,53} Due to its variable carotenoid content and the high energy cost of the drying process, shrimp meal appears to have limited potential as a pigment source in commercial salmon farming.

Spinelli and Mahnken³⁴ extracted carotenoids from red crab (*Pleuroncodes planipes*) and shrimp waste (*Pandalus jordani*) by mixing nine parts of comminuted waste with ten parts of water and one part of soya oil containing 0.025% ethoxyquin. To increase the amount of carotenoid in the oil, a three-stage counter-current process was used. An extract very high in astaxanthin (1530 mg/kg) was obtained. This extract produced acceptable pigmentation in coho salmon (*O. kisutch*) after 120 days of feeding when included in the Oregon moist pellet diet at astaxanthin levels of 30, 60, and 90 mg/kg (Table 4). Chen and Meyers⁷⁶ increased the astaxanthin level in an extract of crawfish (*Procambarus clarkii*) waste by 40 to 50% and increased oil recovery by 10% using a similar extraction method after ensiling the waste. Acceptable coloration (Table 4) has also been achieved using pigments extracted from crustaceans using acetone or acetone/methyl dichloride.^{62,66}

Hansen⁵¹ extracted shrimp (*Pandalus borealis*) waste 3 to 4 times with warm soya oil (200°C) and obtained an extract containing 60 to 70 mg astaxanthin/kg. This extract did not produce significant astaxanthin deposition when fed to rainbow trout at a level of 4.8 mg/kg wet feed (65% moisture) in a wet diet for 104 d. The low astaxanthin concentration in the diet may be the reason for this, but heating astaxanthin to 200°C can produce isomers or derivatives which are not absorbed by salmonids.

Norwegian commercial fish oil produced from capelin (*Mallotus villosus*) contains considerable amounts of astaxanthin due to the intestinal contents of capelin, especially during the summer (Table 3). Astaxanthin in this oil is absorbed and deposited in rainbow trout.^{37,51} The value of these extracts as pigment sources is variable, and the pigmentation value is highly dependent upon the extraction method. In most cases, pigment from an extract is a less effective pigmenting agent than the pigment from the original source. Presently, there is a limited availability of commercially produced pigment extracts.

B. Yeast

Savolainen and Gyllenberg⁶⁹ fed *Rhodotorula sarnellii* preparations to rainbow trout and compared pigment deposition to that from a commercial diet containing synthetic

canthaxanthin. The carotenoid composition is shown in Table 3, while the amount in the diet and the amount deposited in the flesh are shown in Table 4. The increased canthaxanthin and lutein levels in the flesh are remarkable since the diet did not contain either of these carotenoids and the consensus is that rainbow trout cannot synthesize or transform other carotenoids into canthaxanthin. The authors did not report increased levels of *R. sarnellii* carotenoids in the fish.

The yeast *Phaffia rhodozyma* contains astaxanthin as its principal carotenoid at 50 to 800 mg/kg, depending on the strain and growing condition ($E_{1\%}^{1\text{cm}} = 1600$ in acetone).^{11,75} Lyophilized freeze-dried yeast cells incorporated into a semi-moist diet at the rate of 15% on a dry weight basis (52.2 mg astaxanthin/kg diet) were fed to rainbow trout for 43 days. This increased their astaxanthin level from 5 mg/kg at the start of the experiment to 10 mg/kg at the end (Table 4).^{11,75}

The high astaxanthin level makes *P. rhodozyma* a possible pigment source for salmonids. It is, however, interesting to observe that the lobster (*Homarus americanus*) did not accumulate astaxanthin from *P. rhodozyma*, suggesting that the yeast astaxanthin configuration (3R-3'R) cannot be an integrated component of the carotenoprotein crustacyanin and therefore is of no value for the lobster.¹¹

C. Plant and Algae

Paprika has a bright red color due mainly to capsanthin [(3R,3'S,5R)-3,3'-dihydroxy- β , κ -caroten-6-one] and capsorubin [(3S,5R,3'S,5'R)-3,3'-dihydroxy- κ , κ -carotene-6,6'-dione], and inclusion of paprika in the diet of salmonids has been reported to pigment their flesh (Table 4).^{2,66,82} It was also found to be a possible pigment source for lobster (*H. americanus*).⁸³

Increased amounts of canthaxanthin and lutein were observed in the flesh of rainbow trout after they were fed diets containing *Hyppophae rhamnoides* oil (Table 4).⁶⁷ This is difficult to explain considering the carotenoid composition of *H. rhamnoides* (Table 3). Similar results were reported by Lee et al.,⁶⁵ who used extracts of the marigold flower (*Tagetes erecta*) and squash flower (*Cucurbita maxima marcia*) in the diet of rainbow trout. The total carotenoid level in the fish increased, and the largest increase was seen in fish fed marigold flower extract (marigold flowers contain low levels of canthaxanthin; Tables 3 and 4). Based on the reported results, products from higher plants seem to have little potential for use as a pigment source in practical diets.

Ketonic xanthophylls, echinenone (β , β -caroten-4-one; Figure 3), canthaxanthin, and astaxanthin occur under favorable conditions as minor components in green algae. Under unfavorable conditions, i.e., nitrogen deficiency, these carotenoids are often synthesized in greater amounts as secondary carotenoids. This ability to synthesize secondary carotenoids is almost exclusively restricted to the subphylum *Chlorophyceae*.⁸⁴ Probably the best known astaxanthin-producing algae is *Chlamy-*

domonas nivalis, which causes red-colored snow in the mountains in the summer.

Algae high in astaxanthin have produced pigmentation in Atlantic salmon when incorporated into dry-pelleted feeds at a level of 78 mg/kg. Kvalheim and Knutsen⁶⁵ reported an increase in the astaxanthin level in the flesh from 0 to 1.2 mg/kg during a 7-month experimental period. This was about 80% of the level in the control group fed a similar diet containing 51 mg synthetic canthaxanthin per kilogram. They claimed that their algae may produce up to 5 g of carotenoid per kilogram dry matter, and that 90% of the carotenoids are astaxanthin esters (87%) and free astaxanthin (3%). No information on species or growth conditions was provided.

It is believed that the low deposition of astaxanthin from the algae compared with the control was due to low absorption of astaxanthin from the astaxanthin esters. The potential for commercial production of astaxanthin-containing algae depends on the capacity to increase the amount of free-astaxanthin or the development of a process for hydrolysis of the astaxanthin esters. The production costs of the algae-derived products must also compare favorably to synthetic astaxanthin and canthaxanthin. The filamentous blue-green algae (phylum: *Cyanophyceae*), *Spirulina spp.*, also contains a high level of carotenoids (Table 3), but Choubert⁶⁶ did not observe pigmentation in rainbow trout fed diets containing this algae (Table 4).

D. Synthetic Pigment Sources

Hoffman La Roche (Basel, Switzerland) started commercial production of synthetic canthaxanthin in 1964 for coloring food and feeds.² This synthetic canthaxanthin is marketed under the trade names "Roxanthin" or "Carophyll red". During the last decade, synthetic canthaxanthin has become the dominant pigment source used to color cultured salmonids. It is readily absorbed and retained compared with other carotenoid sources and produces nearly the same visual coloration of the flesh as astaxanthin (Table 4).^{38-40,47,51} This pigment is available as a stable dry beadlet, containing 10% canthaxanthin, which can be incorporated into any fish diet at controlled levels.

Free astaxanthin ("Carophyll pink") has also recently been synthesized on a commercial scale (Hoffman La Roche). It seems to be absorbed and deposited better than canthaxanthin by salmonids (Table 4) and is currently being added to some commercially produced, dry-pelleted diets.^{32,33,38,45-47,54,70}

In both synthetic astaxanthin and canthaxanthin beadlets, the carotenoids occur as an equilibrium mixture of *cis* and *trans* isomers. Storebakken et al.⁵⁴ reported about 15% *cis* isomers in beadlets of synthetic astaxanthin. The absorbance for *cis* isomers is lower than for *trans* isomers. Consequently, the extinction coefficient ($E_{1cm}^{1\%}$) must be adjusted to account for the *cis/trans* ratio in quantification of carotenoids from beadlets.⁸⁶

V. METABOLISM OF CAROTENOIDS

Retention of carotenoids in animals depends on several interacting factors:

1. The efficiency of absorption from the digestive tract
2. Transport capacity
3. Deposition mechanisms in the various tissues
4. Metabolism and rate of excretion

Rates of retention of dietary carotenoids in salmonids vary with such factors as fish size, sex, species, and diet composition, but are generally in the range of 1 to 18%.^{6-8,45,73} Carotenoids deposited in the flesh of rainbow trout are reported to have a low metabolic turnover.^{75,87} Those studies, however, reported the amounts of carotenoids in the flesh and disregarded the large amounts in skin.

Salmonids selectively deposit different carotenoids in various tissues (Table 2). For example, the ratio of canthaxanthin to astaxanthin in plasma was found to be significantly different from the ratio in the diet.³⁸ In addition, the ratio of canthaxanthin to astaxanthin in the flesh was found to be significantly different from the ratio in the plasma. Those results imply differential transport, deposition, or metabolism of canthaxanthin and astaxanthin.

Results show a higher total carotenoid deposition in the flesh when both astaxanthin and canthaxanthin are administered together in the same diet compared with adding the carotenoids individually (Figure 4).^{47,71} This indicates that the absorption and metabolism of astaxanthin and canthaxanthin are to some extent independent. However, no information is available on the relative importance of each of the factors on the retention of carotenoids.

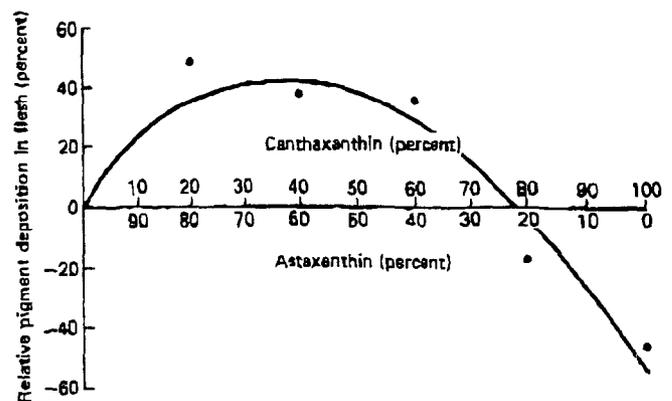


FIGURE 4. Relative pigment deposition in rainbow trout fed diets containing 200 mg astaxanthin + canthaxanthin per kilogram at various astaxanthin-canthaxanthin proportions. (Based on data from Reference 47.)

A. Digestion and Absorption

Absorption of a carotenoid is affected by the digestibility of the raw material in which it is found. This was clearly shown by Johnson et al.,⁷³ who fed the astaxanthin-containing yeast *P. rhodozyma* to rainbow trout. Astaxanthin from whole yeast cells was poorly absorbed (1.5 mg/kg flesh/45 d), while a high astaxanthin level in the flesh was achieved when the cell walls of the yeast had been digested by *Bacillus circulans* prior to addition of the yeast to the diet (11.9 mg/kg/45 d). Similar results were reported by Torrissen et al.²³ They found apparent digestibilities of astaxanthin from fresh shrimp waste and from shrimp-waste meal to be 45% and that from shrimp-waste silage to be 71%. The increased digestibility in shrimp-waste silage was attributed to degradation of the chitin/calcium/protein matrix that binds the pigment within the shrimp shell.

Large variations in the apparent digestibility of synthetic astaxanthin, astaxanthin dipalmitate, and canthaxanthin in diets fed to Atlantic salmon, rainbow trout, and sea trout have been reported (Table 5).^{22,23} The variations were attributed to incomplete extraction of the feces, destruction of carotenoids in the intestinal tract, or destruction of carotenoids during storage. From those data, it seems that the digestibility of carotenoids is less efficient than that of the major nutrients. However, the relatively low digestibility does not explain the low retention of dietary carotenoids.

Table 5
Digestibility of Carotenoids by Rainbow Trout, Atlantic Salmon, and Sea Trout

Carotenoid	Rainbow trout ^a	Atlantic salmon ^b	Sea trout ^a
Astaxanthin	91—97	45—74	74—96
Astaxanthin dipalmitate	42—67	39—52	13—20
Canthaxanthin	45—71	57—67	20—72

^a The diets contained 30 mg canthaxanthin/kg + 30 mg astaxanthin dipalmitate/kg.²²

^b The diets contained 30, 60, 90, mg/kg of the respective carotenoids.²³

In the past, carotenoids were assumed to be physiologically inert compounds that were carried into the body by lipids as carriers. Osborne et al.⁸⁸ did not find any correlation between hypocarotenoidemia and lipid malabsorption in chickens and concluded that there are specific processes for the absorption of carotenoids. This was later confirmed by other workers, who showed different sites of absorption for zeaxanthin and lutein in the chicken and for astaxanthin and canthaxanthin in rainbow trout.^{24,89}

Feeding rainbow trout gelatin capsules containing ³H-labeled canthaxanthin gave a large variation in the total blood canthaxanthin level between the individual fish. The time for reaching the maximum level of blood canthaxanthin varied from 8 to 36 h after force-feeding.⁹⁰ This shows that absorption of canthaxanthin is a slow process compared with absorption of fatty acids, but is comparable to tripalmitin absorption and

transport in carp.⁹¹ The total pigment recovery after 72 h was reported to 0.19%, which is low compared with reported apparent digestibility of carotenoids.^{32,33,53}

B. Transport

Increased carotenoid levels in plasma during anadromous migration of chum salmon (not feeding) were observed by Kitahara.²³ Torrissen³⁸ showed the presence of astaxanthin and canthaxanthin in the plasma of feeding rainbow trout, indicating that serum is the transport medium. Nakamura et al.⁹² found that astaxanthin in the serum was bound to the high density lipoprotein (HDL) (Figure 5). In humans, β -carotene is mainly transported by the low density lipoprotein (LDL) (80%) and very low density lipoprotein (VLDL) (12%) fraction.⁹³ Nakamura et al.⁹² also noted a small shift in the absorption maximum from 478 nm in serum HDL to 470 nm in petroleum ether, indicating that astaxanthin is not covalently bound to HDL in salmonids. It was shown by Ando et al.⁹⁴ that vitellogenin, the precursor of egg yolk protein, might participate in the transport of astaxanthin from the muscle to the ovaries of maturing females (Figure 5). The absorption mechanism in the gastrointestinal tract and the mechanism of transfer of muscle astaxanthin to the skin and gonads via the serum is not known.

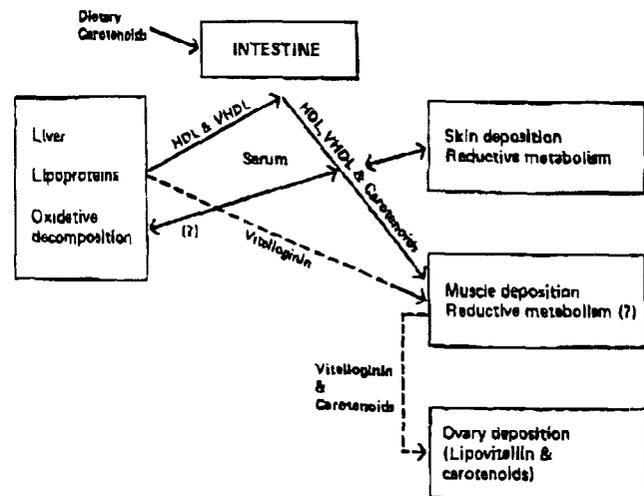


FIGURE 5. Transport and metabolism of carotenoids in salmonids. (Based on data from References 23, 43, 56, and 92.)

C. Metabolization of Carotenoids

Goldfish and fancy red carp (*Cyprinus carpio*) transform ingested zeaxanthin via adonixanthin (3,3'-dihydroxy-4-keto- β -carotene; Figure 3) to astaxanthin, and echinenone to canthaxanthin.^{44,48} A slow transformation of β -carotene to astaxanthin was also shown, but no transformation of the α -lutein structure to the β -structure occurred.

Salmonids are not able to perform these transformations, but the opposite reduction of astaxanthin to zeaxanthin in anadromous migrating chum salmon has been demonstrated (Figure

3).^{23,26,35} Kitahara²³ claimed that this pathway is unique to salmonids, but Schiedt et al.⁴³ disagreed, claiming that ingested astaxanthin is reduced to zeaxanthin in the chicken. They later confirmed the reduction of astaxanthin to zeaxanthin in experiments with rainbow trout and Atlantic salmon. They also reported the transformation of astaxanthin, canthaxanthin, and zeaxanthin to vitamins A1 and A2 in vitamin A-depleted rainbow trout.

The liver is the carotenoid storage and metabolizing organ of poultry.⁴³ In contrast, low levels of oxygenated carotenoids have been found in fish liver.^{26,35,42} Metabolites of carotenoids ingested by fish are found mainly in the skin, but also in the flesh of sexually maturing salmon, while 90% of the deposited astaxanthin is in the flesh.^{23,24,26,43} Astaxanthin and canthaxanthin appear to be degraded in the digestive tract of salmonids.^{32,33} Such degradation could not be detected by feeding ¹⁴C-zeaxanthin to fancy red carp.⁴⁸

VI. DIET COMPOSITION AND CAROTENOID DEPOSITION

A. Dietary Level of Carotenoids

As shown for chickens, the dietary level of carotenoid is a major factor in determining the level of carotenoid in the flesh of salmonids.¹⁸ Despite its importance, little work has been conducted on the effect of dietary carotenoid level on pigment concentration in marketable fish and on carotenoid retention efficiency. Most studies have been conducted using small fish over a short period of time and do not give a comprehensive picture of the interaction of dietary pigment concentration on pigment retention.^{33,34,72,75}

The total body concentration of carotenoids (C_B) may be described by the following equation:

$$C_B = C_F \times F \times (W_1 - W_0) \times R / (W_1 \times 100)$$

where C_F = dietary level of carotenoids (mg/kg); F = feed conversion ratio (kg feed/kg fish weight gain); R = % retention of pigments; W_0 = start weight (kg); and W_1 = final weight (kg).

Two hypothetical models for pigment deposition are shown in Figure 6. The assumptions are

1. Dietary concentration = 50 mg/kg
2. Starting fish weight = 0.2 kg
3. Average pigment retention = 5%
4. Feed conversion ratio = 2 kg/kg fish produced
5. Feed conversion constant through the growout stage

Based on these assumptions, a fish weighing 5 kg will reach a total body concentration of 4.8 mg/kg. A linear increase in carotenoid concentration as the fish increases in weight implies that retention efficiency also increases. In the example, reten-

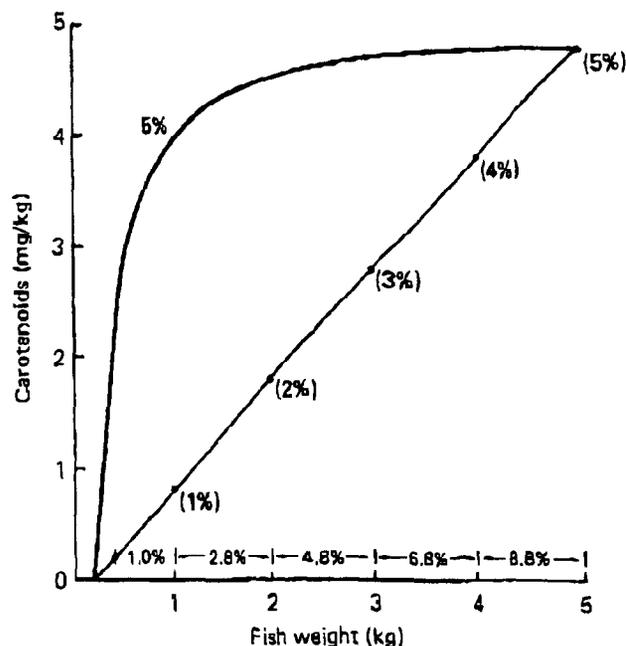


FIGURE 6. Carotenoid deposition in salmonids at an average 5% retention. A linear carotenoid deposition with increasing fish weight implies that the retention increases from 1% for fish weighing from 0.2 to 1.0 kg to 8.8% for fish weighing from 4 to 5 kg. The retention values in brackets represent the average retention at a given fish weight. A constant retention throughout the life cycle (bold line) gives a slower increase in carotenoid concentration with increasing fish weight due to a large flesh to pigment volume.

tion efficiency increases from 1% between 0.2 and 1 kg fish weight to 8.8% between 4 and 5 kg fish weight. Many studies support this linear model, but these studies were conducted over a short time period or with low-growth rates.^{32-34,34,72,75} The results may not be representative of pigment deposition over a whole production cycle. Based on this model, pigmentation of salmonids should start relatively late in the production cycle and the shorter pigmentation time compensated for by a higher level of carotenoids in the diet.

The second model is based on the assumption that pigment retention is constant throughout the life cycle. This model predicts a rapid increase in pigment level for small fish and a plateau in pigment deposition as the fish get larger. This model is supported by data for rainbow trout.^{47,70,95} Storebakken et al.⁷⁰ published the only study where the pigment development of fish from 176 g to about 3 kg was monitored. Those results strongly suggest that, at least for fish above a certain size (0.5 to 1 kg for rainbow trout), pigment retention is fairly constant.

A constant pigment retention rate suggests that pigmentation should be started early in the production cycle and that the level of pigment in the feed determines the final pigment level in the flesh. Based on this assumption, Figure 7 shows expected carotenoid levels in fish of different sizes in relation to dietary pigment concentration and for different pigment retention rates.

The level of dietary carotenoid appears to affect retention

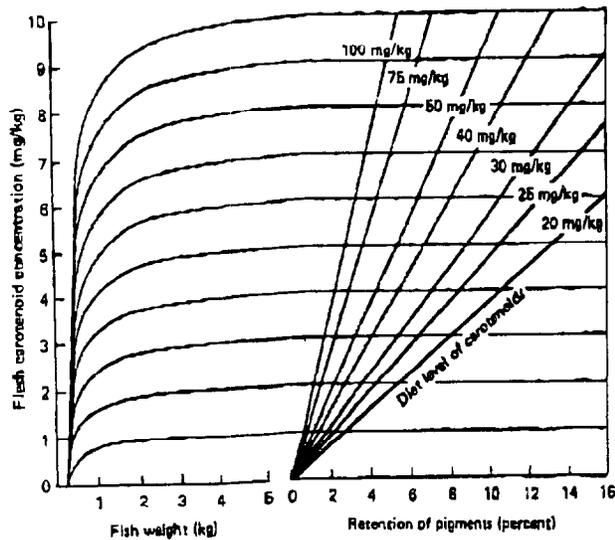


FIGURE 7. Expected carotenoid levels in salmonids at different dietary carotenoid levels, pigment retention, and fish weights. The figure is based on the assumption that feeding starts at a fish weight of 0.2 kg and that the retention is constant throughout the life cycle.

in salmonids.^{33,34,72,75} In Figure 8, the relative pigment deposition is shown as a function of change in the dietary level of carotenoids. The results from Kotik et al.,⁷² Spinelli and Mahnken,³⁴ and Torrissen⁷⁵ were similar, showing a lower relative rate of pigment deposition when the level of carotenoids in the diet increases. It was also found that the apparent digestibility of canthaxanthin in rainbow trout decreased by increasing dietary concentration of canthaxanthin.⁹⁶ Storebakken et al.³³ did not find the same pattern.

The pigment retention in the studies showed extreme variation. Kotik et al.⁷² reported a retention rate of between 20 and 60%, while the results of Storebakken et al.³³ suggested a retention rate in the range of 1.7 to 5%. Hardy and Torrissen⁹⁷ estimated the retention in commercially produced salmonids to be 4 to 5% when the diet contained 50 to 75 mg/kg astaxanthin or canthaxanthin.

B. Dietary Lipid Level and Lipid Source

The effect of dietary lipid level on pigment deposition is not clear. Abdul-Malak et al.⁹⁸ and Choubert and Luquet⁴ observed no significant effect when the dietary lipid level was increased from 9.4 to 17.4%. Seurman et al.⁷⁴ and Torrissen⁷⁵ observed moderate, but significant, increases in astaxanthin deposition, while Spinelli⁷² achieved a 33% increase in flesh astaxanthin by increasing the fat content from 10 to 15% in diets fed to rainbow trout. The apparent digestibility of canthaxanthin in rainbow trout was found to increase by increasing the levels of dietary lipids.⁹⁶

In all of the studies cited, pigment deposition in the flesh was used as an indicator of the effect of dietary lipid level on pigment absorption. Exchanging protein for fat will, up to a

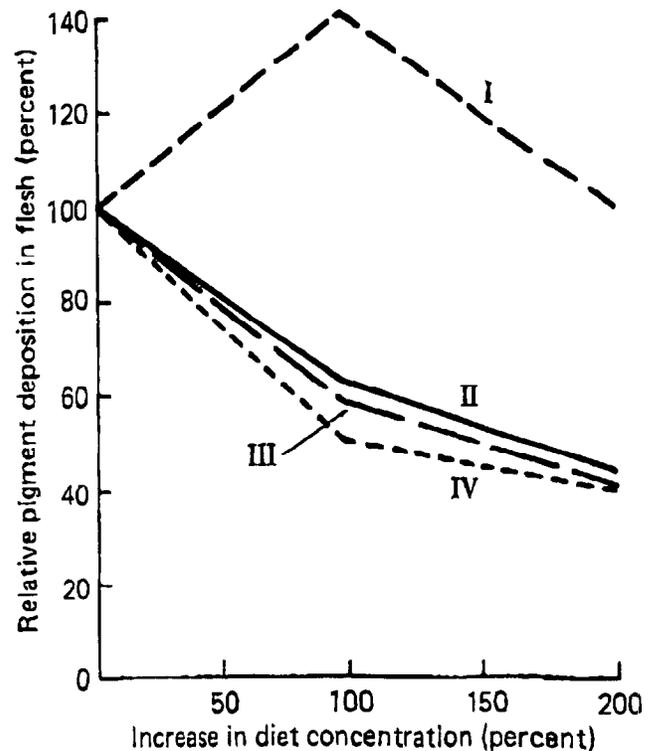


FIGURE 8. Relative pigment deposition in the flesh of salmonids with increasing dietary levels of carotenoids. (Based on data from Reference 33 for I; Reference 75 for II; Reference 34 for III; and Reference 72 for IV.)

certain point, increase the energy level in the diet and give an increased growth rate and fat deposition. Increased growth complicates the evaluation of the results as the actual amount of flesh to be pigmented increases and thereby causes a dilution of the carotenoid level in the flesh.

The effect of dietary fat level on deposition of carotenoids in hen eggs shows a similar disparity. Saunders et al.⁹⁹ in their review put forward the hypothesis that lipids not only promote absorption, but also enhance oxidative degeneration of carotenoids. They suggested that the oxidative effect can be reduced by the addition of antioxidants such as BHT and vitamin E. Torrissen⁷⁵ did not find a significant effect on pigment deposition in rainbow trout when α -tocopherolacetate was added to the feed. However, α -tocopherolacetate does not act as an antioxidant in diets. It is found that frozen farmed salmon has a greater tendency to fade than wild salmon, probably due to oxidation of the carotenoids. Spraying filets from farmed salmon with butylhydroxyanisol (BHA) or ascorbic acid reduces this fading. In this aspect, BHA seems to be more efficient than ascorbic acid. Additional amounts of tocopherol (vitamin E) in the diet did not have the same effect.¹⁰⁰

The lipid levels in commercial dry-pelleted diets for salmonids are in the range of 18 to 22%, and a further increase in the lipid level would cause problems in manufacturing the diet and might also cause unwanted high lipid levels in the flesh. The quantity of canthaxanthin deposited in trout flesh is also

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.¹⁰⁰

VII. PHYSIOLOGICAL FACTORS

A. Fish Size

Rainbow trout and chinook salmon (*O. tshawytscha*) weighing <150 g deposit little canthaxanthin or astaxanthin in the flesh.^{98,101} Spinelli and Mahnken³⁴ and Torrissen⁴⁹ reported a similar effect, but a lower fish weight (80 to 90 g). Other data indicate that Atlantic salmon must be larger, 200 to 400 g, before they deposit carotenoids efficiently.⁴⁶ It was also found that arctic charr (*Salvelinus alpinus*) weighing between 125 and 200 g deposited canthaxanthin more efficiently than fish between 17 and 25 g. The large fish had an apparent digestibility of canthaxanthin of 39% compared with 18% for the small charr. In the large fish, the canthaxanthin was evenly distributed in the fillet, while the caudal musculature in the small fish contained 30% more canthaxanthin than the dorsal musculature.¹⁰²

Why salmonids below a certain weight do not deposit carotenoids in the flesh is not known. Since they accumulate carotenoids in their skin, they obviously absorb dietary carotenoids. Brightly pigmented rainbow trout, brown trout, and Atlantic salmon below these minimum sizes are often found in over-stocked lakes and pens. From these observations, one could conclude that the ability of salmonids to deposit carotenoids is more related to age and physiological status than to actual fish weight or dietary carotenoid level.^{101,103}

B. Sexual Maturation

Mobilization of the carotenoids from the flesh and redeposition in the skin and ovaries during maturation have been reported and quantified by several authors.^{15,23,103,104} This depletion of flesh pigments has important economic implications, being one of the factors limiting the acceptability of maturing salmonids to the consumer.

Spawning fish have only traces of astaxanthin in their flesh compared with 17 to 32 mg/kg in immature fish.¹⁵ This observation was confirmed by others, who reported a decrease in flesh astaxanthin in coho salmon from 6.3 to 6.7 mg/kg in immature fish to 0.4 to 0.8 mg/kg at spawning.^{23,105} Crozier,¹⁰⁴ in his work with sockeye salmon (*O. nerka*), reported that only 1% of the astaxanthin in immature fish was retained after spawning (23 to 28 mg/kg vs. 0.3 mg/kg).

Torrissen and Torrissen¹⁰⁶ detected a decrease in the carotenoid content of the flesh of Atlantic salmon about 6 months prior to spawning. Spawning Atlantic salmon retained more astaxanthin in the flesh than Pacific salmon, approximately 40% compared with 1%. This might be due to the fact that the farmed Atlantic salmon were fed until 2 to 3 weeks prior to

spawning, while Steven,¹⁵ Ando et al.,¹⁰⁵ Kitahara,²³ and Crozier¹⁰⁴ examined wild fish which stop feeding prior to their spawning migration.

The carotenoid level of skin does not show large seasonal variation, but the level increases in both sexes during maturation.^{23,107,108} This increase is greater in males than in females. The skin also contains a larger variety of carotenoid pigments than the flesh and ovaries. At least 16 carotenoids have been isolated from the skin of chum salmon.²³

C. Genetic Factors

As early as 1916, Prince¹⁰⁹ observed differences in flesh pigmentation among and within salmonid species. Storebakken et al.⁷⁰ compared canthaxanthin deposition in the tail flesh of rainbow trout, Atlantic salmon, and sea trout fed dry-pelleted or wet diet containing 40 and 15 mg canthaxanthin/kg at four stations along the Norwegian coast. The level of the carotenoid was highest in rainbow trout (13.7 and 10.2 mg/kg for the dry and wet diets, respectively), followed by sea trout (12.4 and 6.1 mg/kg), and Atlantic salmon (6.12 and 4.12 mg/kg). Corresponding results were shown by Foss et al.,³² who compared astaxanthin and astaxanthin dipalmitate (30 mg/kg dry diet) in diets which also contained 30 mg canthaxanthin per kilogram as a pigment source for rainbow and sea trout. After 22 weeks of feeding, rainbow trout reached a total level (astaxanthin + canthaxanthin) of 10.7 and 10.4 mg/kg when fed free-astaxanthin + canthaxanthin, and astaxanthin dipalmitate + canthaxanthin, respectively. The corresponding values for sea trout were 1.8 and 1.9 mg/kg. However, the results are difficult to interpret due to large differences in growth rate and consumption of carotenoids among the species.

Regression of carotenoid concentration in the flesh (mg/kg) on fish weight gave the following coefficients: rainbow trout, 1.44 and 1.21 for dry and wet diets, respectively; Atlantic salmon, 0.44 and 0.29; and sea trout, 3.71 and 2.12.⁷⁰ Assuming equal feed conversion ratios, sea trout appear to have the highest carotenoid retention, followed by rainbow trout and Atlantic salmon.

Quantitative genetic variation in flesh carotenoid levels has also been described for coho salmon, chinook salmon, rainbow trout, and Atlantic salmon.^{30,31,101,110,111} Choubert and Blanc¹¹² concluded that canthaxanthin-pigmented triploid and diploid rainbow trout do not differ in flesh color, at least in fish which have not begun to mature sexually.

Chinook salmon display a wide spectrum of flesh colors among the "red-fleshed" groups, but there is also a distinct "white-fleshed" form.¹¹³ The "red-fleshed" and "white-fleshed" types may be demonstrating a threshold trait with very high heritability, or Mendelian traits under control of at least two loci, whereas the degree of pigmentation in "red-fleshed" coho salmon, rainbow trout, and Atlantic salmon apparently is a polygenic trait with low heritability.¹¹³

VIII. FUNCTION OF CAROTENOIDS

It is well documented that carotenoids have a photoprotective role in plants.¹¹⁴⁻¹¹⁶ Except for its function as a vitamin A precursor, far less information is available on the biological functions of carotenoids in animals.

Sargent et al.¹¹⁷ noted that extracts of feces from rainbow trout fed *Calanus finmarchicus* were dark red, while extracts from herring (*Clupea harengus*) fed the same material were dark green. Herring, like most other fishes, do not accumulate carotenoids in their flesh in significant amounts, so salmonids absorb and metabolize carotenoids quite differently than herring do. Why salmonids have a unique carotenoid metabolism is not known, and so far no biological function of the carotenoids in salmonid flesh has been documented. It has, however, been shown that carotenoids supplied in the diet to fry of Atlantic salmon increase the growth rate.¹¹⁸ This strongly indicates that they have a biological function. The mobilization of carotenoids, and their transport from the flesh to the skin and ovaries during maturation, has led to the hypothesis that carotenoids have a function in reproduction. Possible carotenoid functions include:

1. Fertilization hormone
2. Source of pigments for chromatophores
3. Function in respiration
4. Protection from light
5. Resistance to elevated temperature and ammonia
6. Provitamin A

These have recently been reviewed and therefore are not covered here.^{9,10}

β -Carotene, and to some extent α - and γ -carotene, and cryptoxanthin are vitamin A precursors.² Gross and Budowski¹¹⁹ claimed that platy (*Xiphophorus variatus*) and guppy (*Lebistes reticulatus*) were able to convert astaxanthin and canthaxanthin to vitamin A. Schiedt et al.⁴³ reported that vitamin A-depleted rainbow trout performed a corresponding transformation, but that vitamin A-sufficient trout only transformed small amounts. In general, the vitamin A supplied from canthaxanthin and astaxanthin must be of minor importance as most of the natural food of salmonids contains vitamin A or β -carotene in relatively large amounts.

β -Carotene and vitamin A have been postulated to have a cancer-protective role in mammals.^{25,120,121} Bendich and Shapiro¹²² reported that both β -carotene and canthaxanthin increased immune responses in rats. Burton and Ingold²⁵ have shown that β -carotene and other carotenoids behave as antioxidants at low oxygen pressure. They concluded that carotenoids may play an important role in protecting lipid tissues from peroxidation *in vivo*. This would explain both the cancer-protective role and also the increased immune response observed. Since carotenoids are effective at low O_2 concentra-

tions, they may complement vitamin E which is effective at higher O_2 concentrations. Cold water fishes, like salmon, have a high level of polyunsaturated fat in their membranes, and protection of lipid tissue from peroxidation seems to be a possible function of astaxanthin or canthaxanthin in salmonids.

IX. CONCLUDING REMARKS

A majority of studies on pigmentation of salmonids have concerned testing of different carotenoid sources on relatively small-sized salmonids over short periods (1 to 4 months). Since many of these sources show considerable variation in composition from batch to batch, it is difficult to obtain reproducible results. Therefore, the value of these studies is limited to providing information on the availability of the carotenoids in specific batches of carotenoid products.

The retention of pigments in salmonids is low, and increasing the retention has great economic importance. It is estimated by Hardy and Torrissen⁹⁷ that increasing carotenoid retention from the approximately 4% in commercial farming today to 10% could produce a potential saving in feed costs of approximately \$10 million U.S./100,000 tons of fish produced. To increase the retention of carotenoid pigments in salmonids, better basic knowledge of factors affecting absorption, deposition, and metabolism are needed for each species throughout its life cycle. Of fundamental importance is a better understanding of the effects of diet composition, fish size, growth rate, length of feeding, and the metabolic turnover of carotenoids on the retention of carotenoids at various dietary concentrations.

The importance of knowing the functions of carotenoids and their dietary requirement is evident, and investigation of possible biological functions of carotenoids in fish at all stages of development should be intensified. Documenting biological function also has important implications in marketing the final product. The regulations on use of color additives in most countries has placed stringent regulations on both their use in fish diets and the marketing of the final products (i.e., the required declarations). Classification of synthetic and natural carotenoids as required nutrients would simplify the addition of carotenoids to fish diets.

Further progress in this field will also require standardization of analytical methods to identify and quantify carotenoid pigments and standardization of experimental designs. Salmonids show large individual variations in their ability to deposit carotenoids, and future studies must utilize uniform experimental fish to reduce this source of variation.

ACKNOWLEDGMENT

This work was supported by the Norwegian Fisheries Research Council (NFFR) and the Directorate of Fisheries. With-

out their financial support to O. J. Torrissen during his stay at the Northwest and Alaska Fisheries Center, Utilization Research Division, this work would not have been possible. We also thank Mr. John Spinelli for support and valuable comments.

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