Pure & Appl. Chem., Vol. 57, No. 5, pp. 685–692, 1985.
Printed in Great Britain.

Absorption, retention and metabolic transformations of carotenoids in rainbow trout, salmon and chicken

Katharina Schiedt, F.J. Leuenberger, M. Vecchi and E. Glinz

Department of Vitamin and Nutrition Research and Central Research Units,
F. Hoffmann-La Roche & Co. Ltd., CH-4002 Basle

Abstract - Absorption and pigmenting efficacy of astaxanthin, canthaxanthin and zeaxanthin were studied in rainbow trout (Salmo gairdneri, R.). Astaxanthin is the best pigmenter, followed by canthaxanthin and astaxanthin dipalmitate. Metabolites present in the skin were isolated, identified and compared with those in the skin of Atlantic salmon (Salmo salar). The distribution of zeaxanthin and astaxanthin in the body of the chicken was also studied comparatively. Emphasis was laid on the configurational analysis of the administered carotenoids as well as of their metabolites. Absorption and deposition of the single stereoisomers were compared. No epimerization of astaxanthin at C(3)/C(3') was detected in rainbow trout, nor was there one in chicken after zeaxanthin administration. A biological function of astaxanthin, canthaxanthin and zeaxanthin as vitamin A (retinol and 3,4-didehydroretinol) precursors was found in vitamin A depleted rainbow trout.

INTRODUCTION

Synthetic carotenoids are of increasing importance for the pigmentation of farmed fish, broilers and egg yolk. In salmonids, astaxanthin is the main natural pigment responsible for the typical pink colour of the flesh. In the chicken and in egg yolk, the natural pigments are lutein and zeaxanthin if the animals are fed in the open on natural feed resources, such as grass and maize.

In recent years, due to improved analytical methods, it has become possible to determine not only minute quantities of these oxygenated carotenoids, but also the configuration of the hydroxy group at C(3) and C(3'). In our laboratories, pigmenting trials with various carotenoids were carried out in rainbow trout (Salmo gairdneri R.) and in chicken. The aim of our investigations was to determine the retention and distribution of carotenoids in the body of the target animals, using 15,15'-$^{3}H_2$-labelled compounds, possible metabolic transformations and epimerization at the chiral centres of C(3) and/or C(3').

It is well known that fish and birds utilize the oxygenated carotenoids for the pigmentation and decoration of their skin and plumage, respectively. In young animals, the dietary carotenoids are also deposited in the flesh. With sexual maturity, however, the carotenoids are mobilized from the flesh and transferred to the reproductive organs and eggs. Various authors have reported on a favourable influence of carotenoids on the fertility and reproduction (Refs.1-4), although no specific biochemical function is yet known. In the literature, xanthophylls are discussed as possible vitamin A_2 (dehydroretinol) precursors in fresh water fish, where vitamin A_2 predominates over A_1 (retinol). In our experiments, e.g., a ratio of A_1:A_2 of approximately 1:4 was found in liver and intestine of rainbow trout. Various authors claim lutein to be a precursor of dehydroretinol in some fresh water fish (Refs.5-9). Gross et al. found evidence of a conversion of astaxanthin, canthaxanthin and isozeaxanthin via ß-carotene to vitamins A_1 and A_2 in guppies and platies (Ref.10). Therefore, the second aim of our studies on ^{3}H-labelled astaxanthin, canthaxanthin and zeaxanthin was to follow up a possible formation of retinol and dehydroretinol in rainbow trout.

GENERAL REMARKS

The deposition rate of carotenoids in organs and tissues depends on the selective absorption through the intestinal wall, on the one hand, and on the utilization and excretion on the other. The different groups of animals are different carotenoid selectors: mammals, e.g., have a preferred absorption of ß-carotene, while fish and birds absorb the 3,4-oxygenated xanthophylls much better.

In salmonids, the oxycarotenoids are deposited in the flesh in the free form, while in skin, predominantly esters are found. In this presentation, not only quantitative aspects of carotenoid absorption and metabolism in single trials will be shown, but also qualitative parallelism and divergencies among different trials, groups and species of animals.

FEEDING TRIALS IN RAINBOW TROUT (SALMO GAIRDNERI, R.) WITH VARIOUS CAROTENOIDS

In a number of trials, rainbow trout were fed various oxygenated carotenoids during 4-7 weeks. In all experiments, the pigments were added to the basic feed as water dispersible beadlets. The carotenoid content found in flesh (0.3-7 µg/g) and skin (5-31 µg/g) show clearly the preferred absorption of 3,3'-dihydroxy-4,4'-dioxo-ß,ß-carotene (astaxanthin) followed by 3,3'-dihydroxy-4-oxo-ß,ß-carotene (adonirubin) and 4,4'-dioxo-ß,ß-carotene (canthaxanthin). The 3,3'-dihydroxy-ß,ß-carotene (zeaxanthin) was absorbed very poorly and the unsubstituted ß-carotene hardly at all. The flesh of untreated trout contains negligible amounts of yellow xanthophylls (0.2 µg/g, lutein and zeaxanthin). In skin and fins, yellow xanthophylls (5-10 µg/g tissue) were found, predominantly 3'-epilutein and zeaxanthin besides traces of lutein, the bulk of them esterified. In pigmentation trials, the administered carotenoid accumulated in the skin without greatly changing the content and the composition of the yellow xanthophylls present also in the untreated control fish.

Astaxanthin

Retention and distribution in the body. (3S,3'S)-Astaxanthin-$^3H_2$ was fed to trout during four weeks at a concentration of 100 ppm in feed: The deposition rate in the whole fish was 10 % as determined by means of the radioactivity in four individuals. The bulk of it, 40-80 %, was found unchanged in the muscle, 12-60 % was present in the skin, dominantly esterified, partly metabolized. The highest concentrations were found in bile (20-40 µg/g), in skin and fins (5-35 µg/g), in operculum (10-35 µg/g) and in the ovaries (7-15 µg/g).

Qualitative analysis of the radioactivity was conducted after extraction of the following target organs and tissues: In bile and blood, which must be considered as vehicles for the transportation of dietary carotenoids, and in liver and pyloric caeca, where part of the digestion takes place, free astaxanthin was found exclusively.

Pigmentation with individual configurational isomers. The individual optically active isomers (3R,3'R)- and (3S,3'S)-astaxanthin and the so-called "racemic" optically inactive mixture of the three isomers (3R,3'R), (3R,3'S) and (3S,3'S) in a ratio of 1:2:1 were fed during five weeks (100 ppm in feed). The respective astaxanthin dipalmitates were also included in the experiment. The configurational isomers of astaxanthin were determined after derivatization to the (-)-dicamphanates and HPLC separation (Ref.11). The results are compiled in Table 1.

Table 1. Content of total carotenoids in flesh and skin and configurational analysis of astaxanthin (20 fish per group, 200-250 g)

Experimental Groups	Flesh				Skin			
	Carotenoid content ug/g	Configurational Analysis RR	RS	SS	Carotenoid content ug/g	Configurational Analysis RR	RS	SS
RR-AXN	5,8	98,7	1,3	0	27,3	97,9	1,4	0,7
SS-AXN	4,7	0,5	2,2	97,4	29,6	0,9	2,4	96,7
"rac." AXN	4,8	24	50	26	27,0	-	-	-
RR-AXN dipalm.	2,3	96,3	2,0	1,3	19,6	94,6	3,3	2,1
SS-AXN dipalm.	1,2	6,2	0,9	93,0	13,7	8,5	1,7	89,8
"rac." AXN dipalm.	3,8	44,4	46,1	9,5	13,8	44,4	46,4	9,5

- Equal absorption/deposition of "racemic" and (3S,3'S)-astaxanthin and a slightly increased deposition of (3R,3'R)- compared with (3S,3'S)-astaxanthin was noted.
- No epimerization was found after administration of free (3S,3'S), (3R,3'R) and "racemic" astaxanthin which is in agreement with trials carried out in Norway by Foss et al. (Ref. 12).
- Astaxanthin esters were generally less utilized than the free ones. (3R,3'R)-Astaxanthin dipalmitate is utilized better than the (3S,3'S) if administered as single isomers, as well as from a "racemic" mixture of the three isomers. (3R,3'R)- astaxanthin accumulated in skin and flesh after administration of the "racemate". This may be attributed to stereospecific ester hydrolases in the intestinal wall. The re-esterification in the skin

was found to be unspecific as to the configuration at C(3)/C(3'). Determination of the acyl⁻ in astaxanthin esters showed mainly fatty acids of C 20:5 and C 18:1.

Metabolism

As mentioned above, metabolites of astaxanthin were observed in skin only. They were isolated and identified by means of HPLC, UV/VIS, partly by MS and NMR. After radio-dilution with the respective synthetic compounds and re-crystallization until a constant specific radioactivity was obtained, the following specific radioactivities could be calculated for the biological compounds:

	dpm/µg	
Astaxanthin	92'100	
ß-Adonixanthin	73'000	
Zeaxanthin	35'000	(ex monoesters)
Zeaxanthin	11'400	(ex diesters)
Zeaxanthin-5,6-epoxide	20'000	
Deepoxyneoxanthin	40'000	(consistent with UV/VIS and MS only)
Lutein	200	

The elimination of the 4-oxo group could be confirmed in feeding trials with non-radioactive canthaxanthin, where echinenone and ß-carotene could be identified and with adonirubin, where asteroidenone was an intermediate to cryptoxanthin. No elimination of the 3- hydroxy group was detected (Fig. 1).

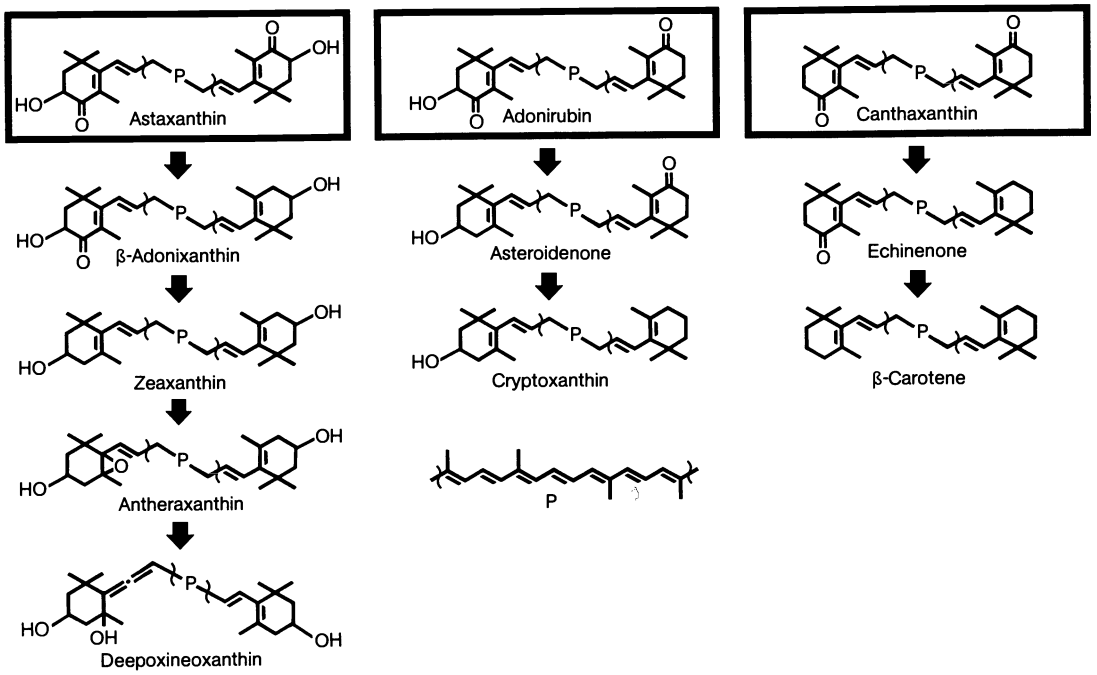


Fig. 1. Metabolic pathway of astaxanthin, adonirubin and canthaxanthin in rainbow trout.

Quantitative evaluation of skin carotenoids after astaxanthin administration yielded the following distribution:
70-75 % Astaxanthin, predominantly diesters and monoesters
c. 15 % Astaxanthin metabolites
c. 15 % Yellow xanthophylls, non-radioactive, as present in negative controls too

Content and configuration of the naturally occurring xanthophylls in the skin of untreated and that of pigmented trout

In all feeding experiments, astaxanthin and other keto-carotenoids accumulated in the skin, but the absolute content and the relative % composition of the yellow xanthophylls, present also in the negative control fish, remained the same. The main components were 3'-epilutein, lutein and zeaxanthin besides small amounts of diatoxanthin and some 5,6-epoxides. Configurational isomers of xanthophylls were determined after derivatization to a dicarbamate and separation by HPLC (Ref.13).

Table 2. Content, composition and configuration of xanthophylls in skin and flesh of rainbow trout pigmented with astaxanthin and negative controls

Feeding trial / Groups / Xanthophylls	Skin						Flesh	
	200 µg/g feed		100 µg/g feed, 5 weeks					
	Negative Control	"Rac." AXN	RR-AXN	SS-AXN	RR-AXN Dipalm.	SS-AXN Dipalm.	RR-AXN Dipalm.	SS-AXN Dipalm.
µg/g tissue								
Astaxanthin	0	19	23.8	26.1	16	9.6	1.7	0.6
Yellow Xanthophylls	10	7.3	3.5	3.5	3.6	4.1	0.6	0.6
Relative Area %								
Lutein[1]	8.3	4.9	4.9	5.4	5.3	7.4	65	64
3'-Epilutein[2]	59.4	65.4	48.8	55	51.4	46.6	4	4
Zeaxanthin	32.3	29.6	45.1	37.6	41.8	44.0	31	32
Zeaxanthin (3R,3'R)	100	56	73	98	79	98.0		
Zeaxanthin (3R,3'S)	0	19	1.7	1.6	1.8	1.8		
Zeaxanthin (3S,3'S)	0	23	24	0.6	18.0	0		

1) (3R,3'R,6'R)
2) (3R,3'S,6'R)

The fact that 3'-epilutein is the major carotenoid in skin, while lutein was found in flesh, should also be considered. It must be assumed that dietary lutein is first deposited in the flesh and possibly epimerized in the skin to the 3'-epilutein. A mere C(3) epimerase can be excluded, since neither astaxanthin nor zeaxanthin was epimerized. Therefore, lutein is likely to be converted via 3',O-didehydrolutein and successive reduction as reported on xanthophyll conversion in the chicken (Ref.15). In a separate feeding trial with natural lutein esters isolated from dried tagetes petals, 3',O-didehydrolutein could be identified from the skin of rainbow trout. Its configuration was (3R,6'R) according to CD. The formation of 3'-epilutein(3R,3'S,6'R) indicates a stereospecific reduction of the C(3')-carbonyl.

An interesting finding is the presence of (3S,3'S)-zeaxanthin in the skin of astaxanthin pigmented fish. In higher plants, hitherto only (3R,3'R)-zeaxanthin has been found. As shown above, in trials with radioactively labelled substances, zeaxanthin was proved to be a direct metabolite of astaxanthin. Astaxanthin itself is not epimerized at C(3)/C(3') during absorption. Moreover, the elimination of oxygen does not alter the configuration at C(3)/C(3'), thus resulting in the corresponding configurational isomers of zeaxanthin. Consequently, the occurrence of the (3S,3'S)- and meso-zeaxanthin, so far considered as unnatural, is likely in aquatic animals, where these have to be understood as metabolites of the different configurational astaxanthin isomers.

CAROTENOID PIGMENTATION IN ATLANTIC SALMON (SALMO SALAR)

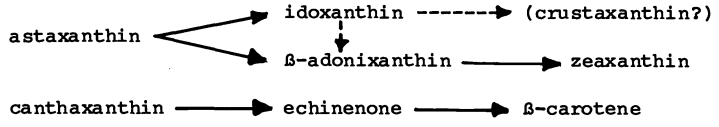
Comparative data on metabolism of astaxanthin, astaxanthin dipalmitate and canthaxanthin

The feeding trials were carried out in Norway by Foss and Storebakken and their collaborators (Ref.14). Detailed results on pigmenting efficacy will be presented by the Norwegian group. Our task was to investigate the metabolites in skin, comparatively with those in rainbow trout. In salmon, dietary astaxanthin and canthaxanthin are deposited more efficiently in flesh than in skin, which is in contrast to the rainbow trout. This experiment was supposed to last approximately one year. The results presented in Table 3 express the values obtained at about half-time and are considered as modest pigmentation only.

Table 3. Carotenoids in skin of Atlantic salmon after pigmentation with "racemic" astaxanthin and canthaxanthin, carotenoid concentration in feed: 90 ppm, duration of feeding: 6 months

Experimental Groups	Neg. Controls	Astaxanthin	Astaxanthin Dipalmitate	Canthaxanthin
Total Carotenoids	1.9 µg/g	5.3 µg/g	4.2 µg/g	3.8 µg/g
Xanthophyll diesters	83 %	30 %	40 %	20 %
Xanthophyll monoesters	17 %	15 %	20 %	6 %
Astaxanthin esters	-	35 %	25 %	-
Adonixanthin esters	-	5 %	5 %	-
Idoxanthin, triol	-	10 %	3 %	-
Canthaxanthin	-	-	-	20 %
Echinenone	-	-	-	20 %
beta-Carotene	-	-	-	25 %
4-Hydroxy-4'-keto-beta-carotene (?)	-	-	-	6 %
Xanthophyll Diesters Saponified				
Lutein	4.1 area %	1.3 area %	1.7 area %	2.3 area %
3'-Epilutein	82.2 area %	60.5 area %	53.5 area %	87.5 area %
Zeaxanthin	13.8 area %	38.2 area %	44.8 area %	10.2 area %
Zeaxanthin SS	17 %	47.7 %	54.8 %	
Zeaxanthin RS	4.4 %	35.7 %	32.2 %	not analyzed
Zeaxanthin RR	78.6 %	16.6 %	12.0 %	

Nevertheless, quantitation and identification of the various carotenoids allow the conclusion that the same metabolites are formed as in rainbow trout. Again, the presence of an increased amount of (3S,3'S)- and meso-zeaxanthin proves that they must be metabolites of the administered "racemic" astaxanthin. A new finding is the presence of idoxanthin, undoubtedly also a metabolite of astaxanthin. This metabolic pathway is in agreement with that found in Oncorhynchus keta by Kitahara (Ref.16). The question arises whether this second pathway of reductive astaxanthin degradation is specific for Atlantic salmon or whether the same mechanism is responsible for the elimination of the 4-oxo groups in rainbow trout. In fact, idoxanthin has never been detected in trout skin, but this might be a question of enzymatic reaction kinetics. Analogously to the studies in rainbow trout, canthaxanthin was converted to echinenone and ß-carotene.



CAROTENOIDS AS VITAMIN A PRECURSORS IN RAINBOW TROUT

As seen in the previous expositions, a constant and relative high content of yellow xanthophylls is maintained in the skin of rainbow trout, although the basic feed contained not more than c. 4 ppm lutein and zeaxanthin. This is all the more surprising as lutein and zeaxanthin are 10-20 times less absorbed than the 4-oxo carotenoids. On the other hand, zeaxanthin and possibly lutein are metabolites of astaxanthin.

What is the biological significance of the hydroxylated carotenoids? Is the transformation of lutein via anhydrolutein, which is then split into 3-hydroxyretinol and dehydroretinol (A_2) as found in some fresh water fish by Barua et al. and Goswami et al. (Refs.5-9) also valid for rainbow trout? These experiments have all been done without radioactive labelling and the determination of A_1 and A_2 was performed simultaneously by Carr Price reaction. In our studies, labelled 15,15'-$^3H_2$-carotenoids and 6,7-$^{14}C_2$-retinyl acetate were used. Vitamins A_1 and A_2, isolated from the intestinal wall, liver and partly from the retinas, whereby the aldehydes were included, were determined by UV/VIS after base line separation by HPLC (Ref.17). The vitamin A level, both A_1 and A_2, was measured in livers from a live weight of 50-700 g. Below 50 g, almost no vitamin A was found, although the diet was supplemented with vitamin A, but increased dramatically from 200 g onwards. This was the best

time for precursor trials. In younger fish, obviously, vitamin A and carotenoids are very poorly absorbed. Two groups of rainbow trout were used:

I Retinol depleted during three months to one year
II Basic diet supplemented with 8,000 I.U. vitamin A/kg feed

Preparation of the precursors: the water dispersible carotenoid preparations were administered in gelatine capsules three times a week under slight anesthesia (corresponding to a dose of 100 ppm in feed for the same period). Two days after the last application, the fish were killed. No other feed was given during the experiment. Retinyl acetate was dissolved in sunflower oil and also administered in capsules as described above. The following results were obtained:

- All three carotenoids, astaxanthin, zeaxanthin, and canthaxanthin, could be confirmed as vitamin A precursors, of both A_1 and A_2, in rainbow trout.
- The degree of incorporation in the vitamin A fraction depends not only on the size and age of the fish, but also on their vitamin A-status. This became very clear in a trial with astaxanthin in A-depleted fish, near to sexual maturity, where as much as 17 % of the liver radioactivity was found in the vitamin A fraction, showing almost the same specific radioactivity as the precursor, corresponding to half of the molecular specific activity after cleavage at C(15)/C(15'). In contrast, no astaxanthin was converted in the fish with a saturated vitamin A status. These metabolic transformations take place in the intestinal wall, which, in all cases, was investigated too.
- From the fact that A_1 showed always a higher specific radioactivity than A_2, it was concluded that A_1 is the immediate precursor of A_2. This was confirmed by the administration of labelled retinyl-6,7-$^{14}C_2$ acetate and is in agreement with the findings of Hata et al. (Ref.18) and Lambertsen et al. (Ref.19).

This knowledge and the fact that carotenoids with and without 3-hydroxy groups, such as, e.g., canthaxanthin, are converted to vitamin A, lead to the assumption that ß-carotene is the common intermediate to A_1, which is then converted to A_2 by dehydrogenation. So far, ß-carotene has not yet been detected among the metabolites isolated from the skin. This might be explained by its exclusive formation in case of vitamin A-depletion and an immediate transformation to vitamins A_1 and A_2 in the intestinal wall. This hypothesis is supported by the finding that, in lutein feeding experiments, neither anhydrolutein nor 3-hydroxyretinol was found in intestine, liver or skin.

Still unknown is the biological activity and significance of A_2 in fresh water fish. Undoubtedly important is the occurrence of dehydroretinaldehyde in the retinas besides retinaldehyde, both forming visual pigments of different absorption maxima, thus enabling the fish to adapt to various light influences of their habitat by changing their ratio of retinal and dehydroretinal (Ref.20). In our own studies with ^{14}C-labelled retinol and tritiated canthaxanthin, the following distribution of the radioactivity was found in the vitamin A fractions of the retinas in two experimental groups, one A-fed and one A-depleted:

Table 4. Vitamin A fractions isolated from 12 retinas of rainbow trout, spec. activity of precursors: ^{14}C-retinol 111'000 dpm/µg, ^{3}H-canthaxanthin 91'000 dpm/µg

Experimental Group / Isolated	^{14}C-Retinyl Acetate		^{3}H-Canthaxanthin			
	A-fed	A-depleted	A-fed		A-depleted	
	total dpm	total dpm	total dpm	µg	total dpm	µg
A_1	34'430	43'070	150	0.67	2'600	0.43
A_2	11'020	14'110	120	0.69	250	0.78
Retinal$_1$	2'660	5'810	430	0.06	340	0.05
Retinal$_2$	1'960	2'646	60	0.08	1'560	0.09
Total (1-2 ug)	50'020	65'636	760	1.50	4'750	1.35
Recovery of dpm in vitamin A fractions (% of lipid extract)	32	34	7.4		54	

Calculation of the specific radioactivity of the single fractions was not possible because cold carrier material was added in the ^{14}C-retinol trial in order to determine recovery, which was 90-97 % for all fractions. In the ^{3}H-canthaxanthin trial, no cold carrier was added, but the whole sample was submitted to HPLC on a preparatory scale. Only all-trans peaks were considered and the cis peaks disregarded. The figure shows that, in the case of retinol administration, the same incorporation was found in both groups, while again a much higher incorporation of canthaxanthin was noted in the case of vitamin A depletion. These results prove a direct biochemical function of hydroxy-keto-carotenoids in fish. But it should not be disregarded that also the mere pigmentation of the integuments plays an important role in the propagation of this group of animals. Without pigmentation, it would not be assured, because, during spawning time, the coloured fins and skin of the males are necessary for the attraction of the females.

Finally, for comparison purposes, some investigations will be presented on zeaxanthin and astaxanthin in chickens representing a group of animals of a higher evolutionary level.

CAROTENOID PIGMENTATION IN THE CHICKEN

^{3}H-labelled (3R,3'R)-zeaxanthin and (3S,3'S)-astaxanthin in broiler and egg yolk pigmentation

Comparison of deposition and distribution in the body of the chicken and metabolic transformation of the natural egg pigmenter zeaxanthin with astaxanthin, which is no component of their natural diet, allowed the following conclusions: Zeaxanthin is three times better absorbed than astaxanthin. This is reflected in higher concentrations in all tissues and organs, such as, e.g., blood, muscle, liver, fat, skin, feathers. The phenomenon observed already in salmon and trout that the carotenoids are deposited in the flesh and skin of the young animal and transferred to the ovaries with sexual maturity, applies also for the chicken. In the young chick (broiler), the bulk of zeaxanthin is found in the muscle, skin, feathers, liver and blood. In the laying hen, 25 % of the ingested zeaxanthin is eliminated in egg yolk, and ovaries contain approx. 50 % of the zeaxanthin recovered in the body.

The liver, where the highest astaxanthin and zeaxanthin concentrations are reached for both, laying hens and broilers, must be considered as the storing and metabolizing organ, which is in contrast to salmon and trout. Interesting is the fact that the chicken is unable to esterify astaxanthin, while the bulk of zeaxanthin in the liver of the laying hen is esterified and stored. Zeaxanthin is partly metabolized to the (6'S)-dehydrolutein as reported earlier (Ref.15). A corresponding oxidative degradation of astaxanthin could not be observed. In contrast, the ketocarotenoid astaxanthin is degraded reductively to idoxanthin and crustaxanthin relatively fast and eliminated from the liver. This pathway of astaxanthin is identical with that of salmon.

Egg yolk pigmentation with the individual configurational isomers of zeaxanthin, (3R,3'R), (3R,3'S), (3S,3'S) and the so-called racemic mixture (3RS,3'RS) of the three isomers in a ratio of 1:2:1.
Zeaxanthin addition to the basic feed: 8 and 16 ppm.
In a parallel line assay, the superior pigmenting potency of the natural (3R,3'R)-zeaxanthin could be demonstrated significantly. The relative pigmenting potency calculated from zeaxanthin concentrations in egg yolk was:

Experimental Group	relative potency:
(3R,3'R)	100 %
(3RS,3'RS) "racemate"	92 %
(3S,3'S)	86 %
(3R,3'S) meso	37 %

The surprising finding that only 40 % of the meso form (3R,3'S)-zeaxanthin was absorbed in comparison with the two enantiomers (3R,3'R) and (3S,3'S) leads to the assumption of an active transport and to the conclusion that the so-called racemic mixture of all three isomers with a 50 % portion of meso-zeaxanthin must also be less absorbed than the single (3R, 3'R)- and (3S,3'S)-isomers. A decreasing ratio of the (3R,3'S)-isomer and a relative increase of the (3R,3'R)- and (3S,3'S)-zeaxanthin should be expected in egg yolk after administration of the "racemate". However, this is not so. The configurational analysis of the zeaxanthin added to the feed and that isolated from egg yolk show clearly that there is no preferred absorption of one individual isomer from the "racemic" mixture, nor was there an epimerization at C(3)/C(3') of the optically active isomers (3R,3'R) and (3S, 3'S). No explanation for this phenomenon could be found so far. A synergistic effect of the "racemic" mixture must be assumed.

CONCLUDING REMARKS

This presentation of a number of various experiments in two groups of animals may be summarized as follows: The 3,4-oxygenated carotenoids play an important role in salmonids and in chicken. Salmon and rainbow trout absorb carotenoids with 4-oxo-functions much better than those with 3-hydroxy-groups only. The reverse is true in the chicken.

The natural pigment of salmonids, astaxanthin, is not epimerized at C(3)/C(3'), but an accumulation of the (3R,3'R)-enantiomer was noted after administration of the "racemic" (3RS, 3'RS)-astaxanthin dipalmitate. The 4-oxo-groups are eliminated, so that adonixanthin and zeaxanthin are formed from astaxanthin, and echinenone and β-carotene from canthaxanthin. Configurational analysis of xanthophylls opens a new possibility for metabolic studies without ^{3}H- or ^{14}C-labelling. Thus, the ratio of the three stereoisomers of zeaxanthin, in particular the occurrence of meso- and (3S,3'S)-zeaxanthin, allowed the conclusion that these are metabolites of meso- and (3R,3'R)-astaxanthin in Atlantic salmon and rainbow trout.

Astaxanthin, canthaxanthin and zeaxanthin were proved to be precursors of vitamins A_1 and A_2 in vitamin A-depleted rainbow trout. This pathway does not work, if dietary retinol is available which is dehydrogenated to A_2 in the intestinal wall.

In the chicken, the optically active (3R,3'R)-zeaxanthin showed a superior egg yolk pigmenting efficacy compared with its enantiomer and the optically inactive meso form. The meso form itself was absorbed more poorly if administered alone than from a so-called "racemic" mixture of all three isomers. Thus, this lecture has to be concluded with some new knowledge, but also with some unanswered questions. This may stimulate further studies on carotenoid absorption, regulatory mechanisms and metabolism in birds and fish.

ACKNOWLEDGEMENT

We should like to thank Drs. R. Zell and E. Widmer for the synthesis of the astaxanthin isomers, Dr. N. Flück and Mr. N. Moser for the preparation of the labelled compounds, Dr. U. Manz for the formulation of the carotenoids, Mr. T. Storebakken, Mr. K. Streiff and Dr. H. Weiser for conducting the animal experiments, Dr. R. Maurer and Mr. K. Schmidt for numerous HPLC analyses, Dr. G. Englert, Mr. W. Grunauer and Mr. W. Meister for NMR and mass spectroscopy, Drs. K. Bernhard, R.K. Müller and A. Rüttimann for providing synthetic carotenoids, Dr. H.J. Mayer for stimulating discussions and support and Mr. S. Bischof for skilful conduction of the experiments.

REFERENCES

1. J. Deufel, Arch. Fischereiwiss. 16, 125 (1965).
2. J. Deufel, Schweiz. Z. Hydrologie 37, 244 (1975).
3. G.S. Georgiev, Folia Balcanica 2, No. 1, 1 (1975).
4. I.D. Shnarevich and E.G. Sakhenko, Nauchnye Doklady Vyskey Shkoly Biologicheskie Nauki No. 12, 20 (1972).
5. A.B. Barua, H.T. Singh and R.C. Das, Brit. J. Nutr. 30, 1 (1973).
6. A.B. Barua and R.C. Das, Brit. J. Nutr. 33, 319 (1975).
7. U.C. Goswami and A. Barua, Indian J. Biochem. Biophys. 18, 88 (1981).
8. A.B. Barua and U.C. Goswami, Biochem. J. 166, 133 (1977).
9. U.C. Goswami and S. Bhattacharjee, Biochemistry Internat. 5, 545 (1982).
10. J. Gross and P. Budowski, Biochem. J. 101, 747 (1966).
11. M. Vecchi and R.K. Müller, High Res. Chrom. and Chrom. Comm. 2, 195 (1979).
12. P. Foss, T. Storebakken, K. Schiedt, S. Liaaen-Jensen, E. Austreng and K. Streiff, Aquaculture (1984) in press.
13. A. Rüttimann, K. Schiedt and M. Vecchi, J. High Res. Chrom. and Chrom. Comm. 6, 612 (1983).
14. P. Foss, T. Storebakken, E. Austreng and S. Liaaen-Jensen, Abstract, 7th Internat. Symposium on Carotenoids, Munich, 27-31 August (1984).
15. K. Schiedt, G. Englert, K. Noack, M. Vecchi and F.J. Leuenberger, Abstract, 6th Internat. Symposium on Carotenoids, Liverpool, U.K., 26-31 July (1981).
16. T. Kitahara, Comp. Biochem. Physiol., 76B, No. 1, 97 (1983).
17. R. Maurer, Central Research Units of F. Hoffmann-La Roche & Co. Ltd., unpublished.
18. M. Hata, M. Hata and T. Onishi, Tohoku J. Agricult. Res. 24, 197 (1973).
19. G. Lambertsen and O.R. Braekkan, Acta Chem. Scand. 23, 1063 (1969).
20. H.J.A. Dartnall, M.R. Lander and F.W. Munz, "Progress in Photobiology" (Proceedings of 3rd Internat. Congress on Photobiology) p. 203 (1961).