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Recent progress on carotenoid metabolism in animals

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Abstract - The influence of dietary astaxanthin, canthaxanthin and zeaxanthin on the carotenoid content and composition of the oil droplets in chicken retina was investigated. From a "racemic" astaxanthin mixture, the (3S,3'S)-isomer was deposited almost selectively in the retina. Both oxidative and reductive metabolic pathways were followed by all three carotenoids. Astaxanthin, the main carotenoid in avian oil droplets, was obviously formed from both dietary zeaxanthin and canthaxanthin.
- Egg yolk pigmentation was studied in relation to carotenoid structure. Depositon rates and metabolites of some C_{30} and C_{40} carotenoids have been determined. Beta- and ψ-apo-carotenoids with a terminal methyl group in the γ-position were gradually shortened by a type of β-oxidation.
- Various yellow metabolites of astaxanthin have been identified in the prawn Penaeus vannamei and their absolute configurations determined. The 4,4'-oxo groups of astaxanthin were reduced stereospecifically, resulting in (4S,4'S)-tetrahydroxypirardixanthin. The presence of the novel, naturally occurring isoastaxanthin [(6S,6'S)-4,4'-dihydroxy-ε,ε-carotene-3,3'-dione] offered an explanation for a racemization of astaxanthin in vivo, which was proved in Penaeus japonicus after administration of optically active [^{3}H]-labelled astaxanthin.

INTRODUCTION

In the past three years since the last Symposium, numerous publications have appeared which document the importance of carotenoids in the industrial farming of animals. By far the greatest number deal with the biological effects of carotenoids in cattle, poultry and aquaculture and are aimed at improving production and quality of the product. A relatively small number of papers deal with metabolic transformations of carotenoids and with their function on a molecular basis. Since, in this meeting, some of the experts in this field reported on their own investigations or reviewed results obtained on animal carotenoid metabolism, I shall present in this article some studies carried out in our own laboratories. These concern:
- deposition of dietary carotenoids and their metabolites in the chicken retina,
- influence of structure on the rate of deposition of carotenoids and metabolites in egg yolk,
- metabolites of astaxanthin in the prawn Penaeus.

CAROTENOIDS IN CHICKEN RETINA

In Boston, three years ago, we discussed the occurrence of some tissue-specific carotenoids in the avian retina and their possible formation in the chicken embryo (ref. 1). Those studies had emanated from a co-operation with Dr. Brian Davies, University of Wales, Aberystwyth, UK. We had seen that distinct, stereospecific carotenoids were responsible for the colour of the red, yellow and greenish oil droplets located in the photoreceptor cells, the cones. Avian oil droplets were assumed to improve acuity in colour vision and were classified by Goldsmith et al. by microphotometric measurements and by analytical procedures (ref. 2).
In 1984, Bethan Davies showed that labelled zeaxanthin was mobilized from the egg yolk to the retina of the chick embryo (ref. 3).

Deposition and transformation of dietary carotenoids in the chicken retina

Dr. Harald Weiser of the Roche Biochemical Animal Section carried out growth tests by feeding (3R,3'R)-zeaxanthin, "racemic" astaxanthin (i.e. a mixture of the stereoisomers RR:RS:SS = 1:2:1) and canthaxanthin to one-day-old chicks (strain Lohmann) (ref. 4). The control diet contained a minimal amount of 450 IU vitamin A and was virtually free of carotenoids. The experimental groups were fed astaxanthin, canthaxanthin or zeaxanthin in dosages of 36-144 mg/kg feed.

For supporting growth, canthaxanthin and zeaxanthin could replace vitamin A partly or entirely. Astaxanthin was no growth factor and the animals that were fed astaxanthin without vitamin A supplementation became weak and moribund and were sacrificed after 21 days. The chicks of the canthaxanthin and zeaxanthin groups were sacrificed after 39 days, the retinas removed and the retinal carotenoids analysed.

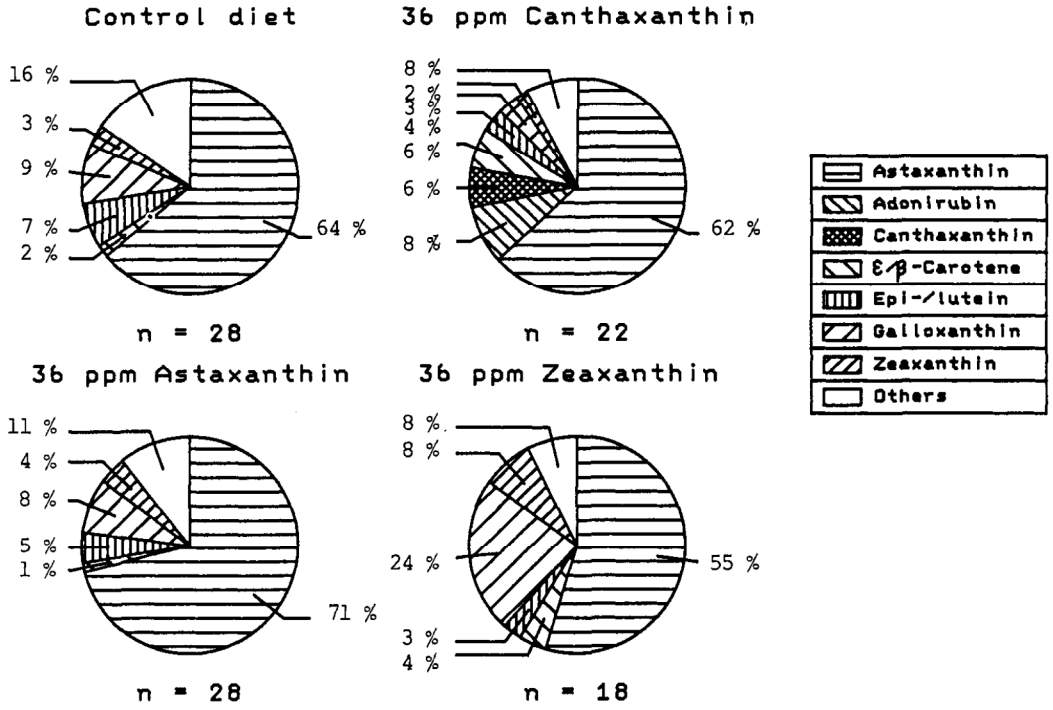


Fig. 1 Carotenoid composition in the retina of chickens fed canthaxanthin, astaxanthin, zeaxanthin or control diet.

Figure 1 presents the relative abundance of carotenoids in the retinas of the four groups that were fed 36 mg carotenoid/kg feed. It is evident that dietary astaxanthin and zeaxanthin did not alter the range of carotenoids present but only changed the ratio of the basic retinal carotenoids, by increasing the amounts of astaxanthin, galloxanthin, zeaxanthin, lutein of various chiralities and of ε,ε-carotene. In the canthaxanthin-fed group, not only were small amounts of this dietary carotenoid found, but also adonirubin and β,β-carotene, which are not usually encountered in the chicken retina (Figs. 2 and 3).

Based on quantitative analyses of the main retinal carotenoids, namely astaxanthin, zeaxanthin and galloxanthin including stereoisomers, the following metabolic processes may be assumed: all three dietary carotenoids were deposited in the retina; astaxanthin was partly metabolized to zeaxanthin and galloxanthin, zeaxanthin increased the amount of astaxanthin and galloxanthin. After administration of astaxanthin or zeaxanthin, a slight increase of ε,ε-carotene was also observed. Canthaxanthin obviously follows an oxidative pathway to astaxanthin as well as a reductive one to β,β-carotene. This is interesting from an evolutionary point of view; apparently, the capability of some crustaceans to transform canthaxanthin into astaxanthin (ref. 5) is preserved in the chicken retina, though this modification is lost in many fishes such as salmonids (ref. 6).

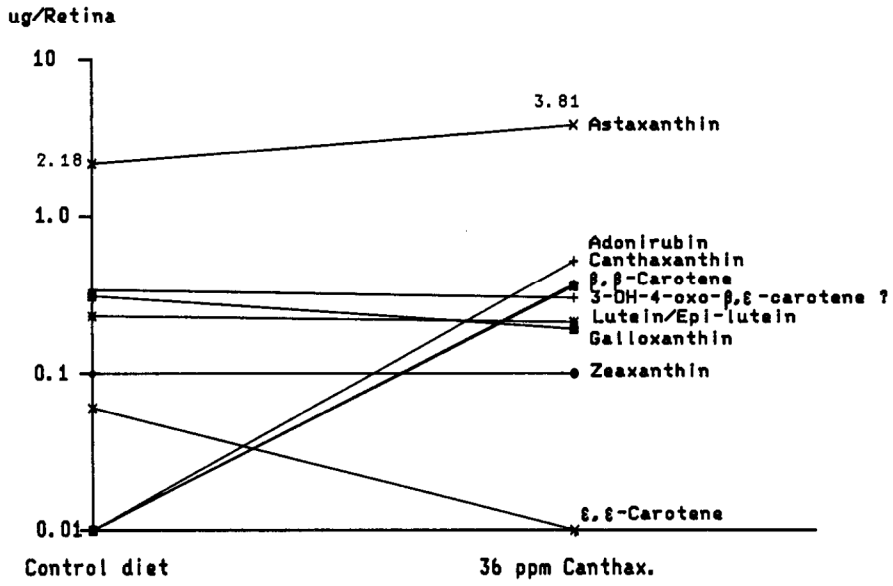


Fig. 2 Carotenoids in chicken retina (µg/retina) after canthaxanthin feeding (36 mg/kg feed) for 39 days

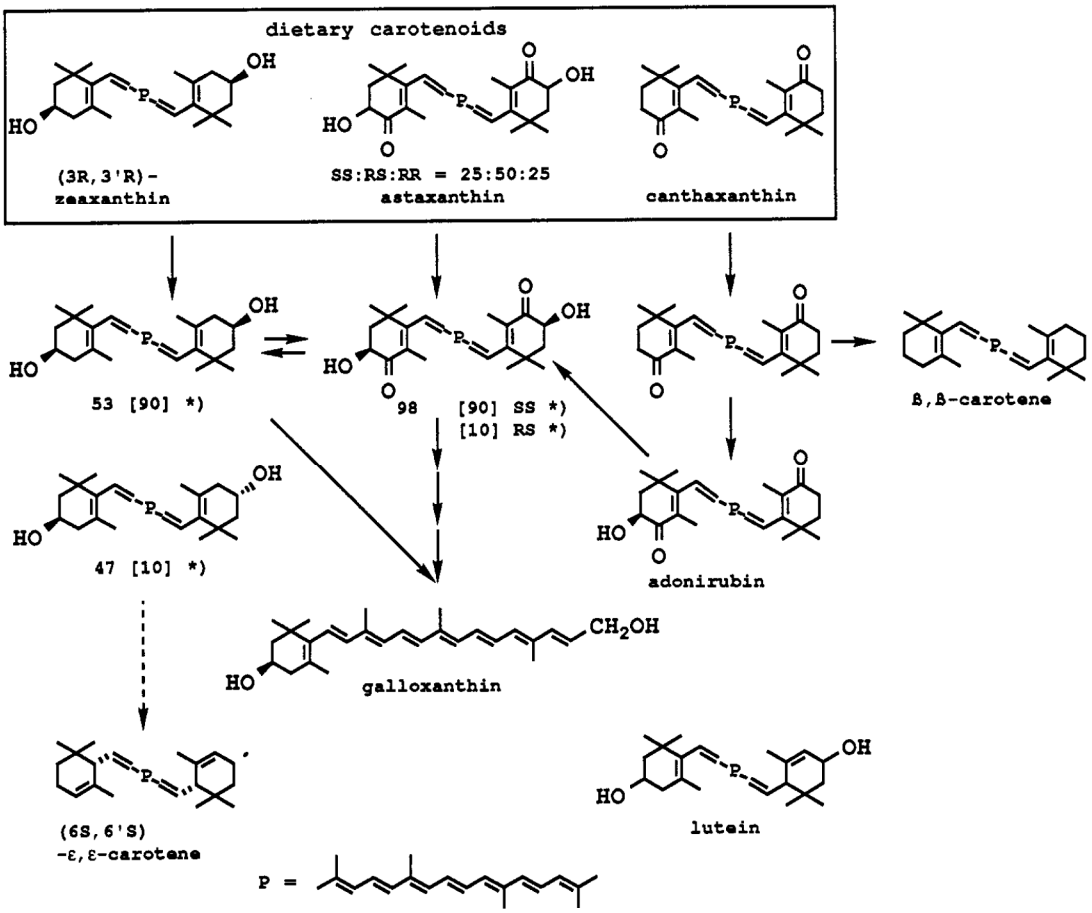


Fig. 3 Deposition and transformation of dietary carotenoids in chicken retina

*) without brackets: Percent of optical isomers in control animals
in brackets [ ]: Percent of optical isomers after carotenoid feeding

Selective absorption of (3S,3′S)-astaxanthin from a racemic mixture and non-racemization of (3R,3′R)-zeaxanthin

These studies may be open to criticism because they were carried out without radio-labelled compounds. However, by means of sophisticated analytical methods, and by isolation of the respective all-trans-isomers and subsequent derivatization (refs. 8, 9, 10) it was possible to determine the quantitative stereoisomeric composition. Table 1 clearly demonstrates that (3S,3′S)-astaxanthin was deposited preferentially, followed by (3R,3′S;meso)-astaxanthin. The (3R,3′R)-isomer from the dietary mixture was not deposited at all. Regarding zeaxanthin, we had, at the last Symposium, discussed the unexpected finding that zeaxanthin in chicken retina was a mixture of the (R,R)- and the meso-isomers. This was intriguing, as zeaxanthin had been considered a precursor of astaxanthin which is optically pure (3S,3′S) (refs. 1, 10). From our experiments, it is now evident that the ingested (3R,3′R)-zeaxanthin was deposited as such; no racemization could be observed. It may therefore be concluded that the meso-isomer is a secondary metabolite of (3R,3′R)-zeaxanthin formed by a redox-system perhaps via 3′-dehydrolutein and lutein (ref. 1, 11). To determine why such an obviously species-specific equilibrium of xanthophyll stereoisomers is maintained in the eye and to establish the possible function of this equilibrium in vision requires further research.

Table 1 Quantity and configuration of astaxanthin and zeaxanthin in the chicken retina after administration of the respective carotenoid

Experimental groups	Astaxanthin in Retina					
		RR		RS		SS
	ng/retina	ng	(%)	ng	(%)	ng (%)
Rac. Astax. *)	6250	0		625	(10)	5625 (90)
Control	4640	0		93	(2)	4547 (98)
Increase in astaxanthin	1610	0		532	(33)	1078 (67)
(3R,3'R)-Zeax. *)	3600	0		72	(2)	3528 (98)
Control	2340	0		42	(2)	2293 (98)
Increase in astaxanthin	1260	0		30	(2)	1235 (98)
	Zeaxanthin in Retina					
		SS		RS		RR
	ng/retina	ng	(%)	ng	(%)	ng (%)
(3R,3'R)-Zeax. *)	500	0		50	(10)	450 (90)
Control	100	0		47	(47)	53 (53)
Increase in zeaxanthin	400	0		3	(1)	397 (99)

*) 36 mg/kg feed

CAROTENOIDS AS POTENTIAL EGG YOLK PIGMENTERS

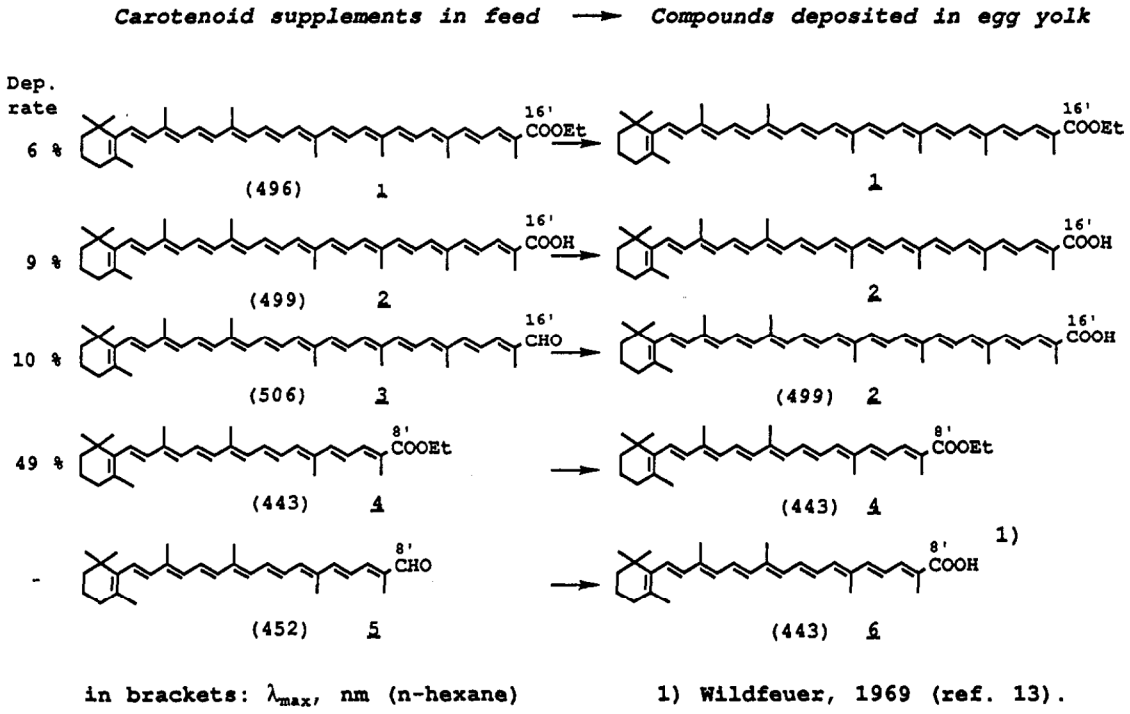
The consumer expects high quality eggs not only direct from the farm but also in industrial poultry products. One mark of perceived egg quality is the yolk colour which depends almost entirely on the carotenoid content and composition in the layers' feed. The appealing appearance of an egg yolk depends not only on the colour hue but also on its saturation and the dominant wavelength that are also responsible for its luminosity.

Influence of carotenoid structure on absorption and deposition

The deposition rate in egg yolk has been tested for some acyclic, cyclic and apo-carotenoids of various structures synthesized by Drs. K. Bernhard and U. Hengartner (ref. 12).

Rather than merely a comparison of pigmenting efficacies by statistical means, some biochemical, analytical studies regarding deposition rate and metabolism have been undertaken. Deposition rate is defined as the amount deposited in egg yolk as a percentage of the quantity ingested. When a compound was converted into metabolites, the amounts of the original compound plus metabolites were summed and considered as total deposition. The aim was to obtain some basic knowledge about how structure influences absorption, deposition and metabolism.
The following compounds were added as beadlets to the basic feed (10 mg/kg) of laying hens (Shaver Starcross 288):
torularhodin ethyl ester 1, *torularhodin* 2, *torularhodin aldehyde* 3, β-apo-8′-carotenoic acid ethyl ester 4, β-apo-8′-carotenal 5, β-apo-2′-carotenal 7, 6′-apo-lycopenoic acid ethyl ester 16, 6′-apo-lycopenal 17, and 8′-apo-lycopenal 18. The pigmentation trial was carried out by Dr. J. Broz of our 'Animal Nutrition' department. Eggs were collected during days 16-20, when the yolk colour had reached plateau values; 20-30 yolks were pooled per group and the administered compound as well as the metabolites were analysed. Numerous chromatographic separations (column, TLC, HPLC) on adsorption and reversed phases were involved as well as chemical reactions and derivatizations such as esterification of the carboxylic acids with diazomethane and finally characterization by MS (Mr. W. Meister) and ^{1}H-NMR (Dr. G. Englert).

The results obtained from a first group, fed C_{40} and C_{30} carotenoids as pigmenting agents are compiled in Fig. 4.



in brackets: λ_{max}, nm (n-hexane) 1) Wildfeuer, 1969 (ref. 13).

Fig. 4 C_{40} and C_{30} carotenoids 1-5 fed to laying hens and compounds deposited in egg yolk

It is known that the C_{40} hydrocarbons β,β-carotene and lycopene are virtually not deposited at all in egg yolk. By the introduction of oxygen functions into the molecule, however, the absorption rate can be improved markedly.

- From the comparison of the red C_{40} carotenoid 1 and the yellow C_{30} apo-carotenoid 4 (Fig. 4) it is evident that the length of the molecule significantly influences the deposition rate in egg yolk. However, the type of the functional group, aldehyde, carboxylic acid or ester, does not change the order of magnitude of the deposition rate.
- The ethyl esters 1 and 4 were deposited as such. Only minor amounts of 3-6% of the total deposited carotenoid had been hydrolyzed to the corresponding acid. The carboxylic acid 2 was deposited unchanged. The aldehydes 3 and 5, however, were oxidized to the respective carboxylic acids.

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