Astaxanthin and Canthaxanthin Kinetics after Ingestion of Individual Doses by Immature Rainbow Trout *Oncorhynchus mykiss*

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Kinetics of a 100 μg oral dose of astaxanthin (AX) or canthaxanthin (CX) was determined from serum AX or CX concentration−time curves in 234 immature rainbow trout, *Oncorhynchus mykiss*, with a mean body weight of 300 g. Carotenoid concentrations in serial serum were measured using HPLC. The net change in mean peak serum concentrations in rainbow trout fed AX or CX reached 4.4 ± 1.0 nmol/mL of serum at 18 h post-dosing for AX and 2.9 ± 0.5 nmol/mL of serum at 12 h post-dosing for CX. The maximum level of CX serum was 1.6 lower than that of AX. In terms of absorption speed, AX and CX are likely equivalent. The relative bioavailability of CX, with respect to AX taken as the reference, corresponds to 33.5%. AX was more slowly removed from the serum than CX. The mean retention time of AX (56.4 h) was 2.4-fold that of CX (23.1 h).

Keywords: *Astaxanthin; canthaxanthin; serum kinetics; pharmacokinetics; rainbow trout*

INTRODUCTION

The pink to red pigmentation of the flesh of salmonids, which is an important criterion for the quality, is due to keto-carotenoids [astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) and canthaxanthin (β,β-carotene-4,4′-dione) (Figure 1)] that fish are unable to synthesize. They must find them in their diet. If in the wild the sources of carotenoids are the natural prey items, in intensive fish farm, salmonids are fed synthetic carotenoid supplemented diets which represent an important part of the production costs.

However, the muscle retention of keto-carotenoids represents only 1−5% of the ingested pigments (Choubert and Luquet, 1979; Torrissen et al., 1990) which may be explained in part by the low digestibilities of canthaxanthin (Choubert and Luquet, 1979) and astaxanthin (Foss et al., 1987). Increasing retention would then enhance the flesh pigmentation and reduce the production costs. An understanding of the carotenoid blood transport will allow for a better utilization of these compounds by fish.

In daily fed fish over a period of 7 days, the maximum level of keto-carotenoids in the serum of rainbow trout was reached within 24 h after the meal (Choubert et al., 1994). In the same way, the absorption of astaxanthin after a single dose of 500 μg in rainbow trout was maximum at 24 h after its administration (March et al., 1990). However, the too large sample time (12−24 h) did not allow more precision.

With this background, the aim of the present study was (1) to assess the level of absorption of astaxanthin and canthaxanthin in rainbow trout fed a single dose of 100 μg of carotenoid and (2) to investigate the pharmacokinetics of the keto-carotenoids in order to determine the absorption and disposition of these compounds in rainbow trout.

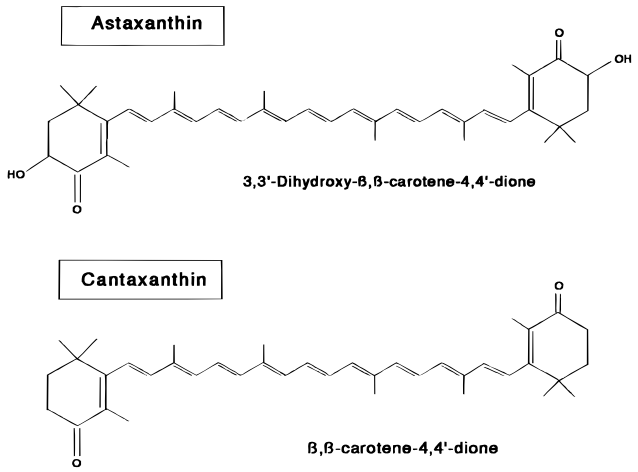

Figure 1. Chemical structures of astaxanthin and canthaxanthin.

MATERIALS AND METHODS

Animals and Diet. A total of 234 rainbow trout (*Oncorhynchus mykiss*) with a mean body weight of 300 g were used as experimental animals. The fish were obtained from the INRA fish farm of Donzacq (Landes department) and kept in recirculated freshwater tanks at 20 ± 0.5 °C during the experimental period (4 days). The photoperiod was maintained at 12 h of light (07.00−19.00) and 12 h of dark (19.00−07.00). The fish were fasted 24 h before the experiment in order to avoid the problem of regurgitation after the anaesthesia.

The test meal contained 100 μg of either astaxanthin (Carophyl pink, 5% astaxanthin, F. Hoffmann-La Roche Ltd, Basel, Switzerland) or canthaxanthin (Carophyl red, 10% canthaxanthin, F. Hoffmann-La Roche Ltd, Basel, Switzerland) put into a little jelly capsule (4 mm in diameter, L = 10 mm, holding capacity 0.13 mL, Coopérative Pharmaceutique

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Française, Melun, France). Fish received only one test diet by force-feeding after anaesthesia (2-phenoxyethanol, 0.4 mL/L, Prolabo, Paris, France) at 9.00 h. One gram of feed [fish meal (45%), soybean oil-cake (20%), pregelatinized starch (26%), corn oil (5%), mineral mix (Labbé et al., 1993) (1%), vitamin mix (Labbé et al., 1993) (1.5%), sodium alginate (1.5%)] without carotenoid was given with the capsule. Then fish were fed each day, once a day at 9.00 h ad libitum, the feed without carotenoid.

Sampling and Analysis. Blood sampling was carried out on 10 anaesthetised fish with 2 mL non-heparinized disposable syringes fitted with 0.6 × 25 mm disposable needles in the caudal peduncle (Le Bail et al., 1981) 3, 6, 9, 12, 18, 24, 30, 36, 42, 48, 72, and 96 h after the test meal ingestion. Bled fish were then put in another tank so that fish had not been sampled several times. Blood samples were protected from light, allowed to clot at room temperature, and held overnight at 4 °C.

At 9, 12, 24, and 36 h after feeding the test diet, five fish were killed by stunning, the digestive tract was dissected out, and the intestine and the liver were separately frozen in liquid nitrogen (−180 °C) and then kept at −80 °C until analysis.

Carotenoid analyses were carried out in duplicate, and the general precautions recommended for isolation and handling of carotenoids (Fiasson et al., 1969) were followed. Carotenoids were determined by HPLC method (Guillou et al., 1993) using a M2200 pump and a lambda 1000 detector (Bischoff, Leonberg, Germany). A guard column (30 × 2.1 mm i.d. stainless steel tube) was placed just in front of the analytical column (250 × 4.6 mm i.d. stainless steel tube). Column and gard column were packed with C_{18} reversed phase material of 5 μm particle size (LiChrosorb, Merck, Darmstadt, Germany). Elution was performed with an isocratic solvent acetonitrile:dichloromethane:methanol:water:propionic acid (71:22:4:2:1, by volume) at 1.0 mL/min. All solvents used were of HPLC grade (Farmitalia Carlo Erba, Milan, Italy) and filtered through a 0.5 μm membrane filter (Bischoff, Leonberg, Germany) before use. The column and the mobile phase were thermoregulated at 3 °C by a recirculating system to avoid variations in peak height and retention time caused by ambient temperature changes. External standards in solutions of known concentrations were used for peak identification and quantification. The detection of carotenoids was performed at 480 nm (astaxanthin) and 472 nm (canthaxanthin). Data were generated in the Pic3 software (ICS, Toulouse, France).

Pharmacokinetic analysis was applied assuming a one-compartment model with first-order absorption and lag time using the nonlinear least-squares program derived from Yamaoka et al. (1981). Noncompartmental parameters, especially areas under the concentration−time curve (AUC) and mean retention times (MRT) of carotenoids, were estimated by using statistical moment theory. Other standard pharmacokinetic parameters were determined by using computer-generated primary coefficients (Gibaldi and Perrier, 1982).

Data were subjected to analysis of variance and *t*-test as appropriate (SAS, 1985). Group differences were considered statistically significant at a level of $p < 0.05$. Data are presented as mean ± SEM.

RESULTS

It should be noted that of 234 fish dosed, four fish for astaxanthin and five fish for canthaxanthin did not show a serum response to carotenoid pigments. Data from these animals were not included in this study.

The net change in mean peak serum astaxanthin concentrations in rainbow trout fed astaxanthin in the water-soluble beadlet form reached 4.4 ± 1.0 nmol/mL of serum at 18 h post-dosing (Figure 2). After 18 h, serum concentrations decreased slowly. At 48 h, concentrations were still at 50% of the maximum serum value, and at 72 h, concentrations were at 30% of the maximum serum value. Statistical analysis revealed no significant ($p > 0.05$) difference between 9 and 48 h. Serum concentrations decreased afterward. Nevertheless the analysis showed great individual variabilities among the different animals.

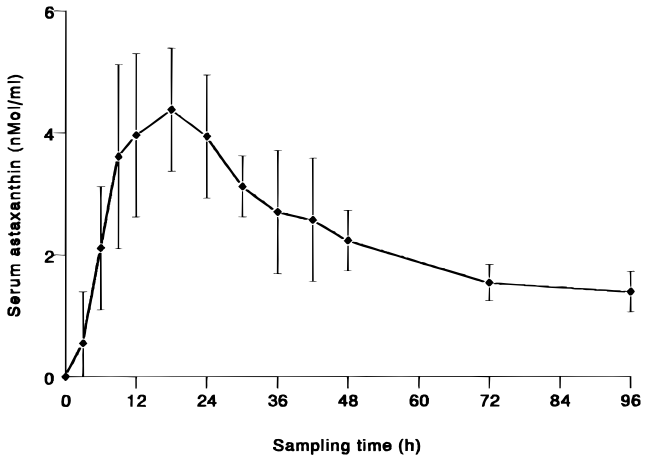

Figure 2. Serum astaxanthin concentration in rainbow trout following a test meal containing 100 μg of astaxanthin in the form of 5% water-soluble beadlets. Values are expressed as means of 10 independent determinations ± SEM.

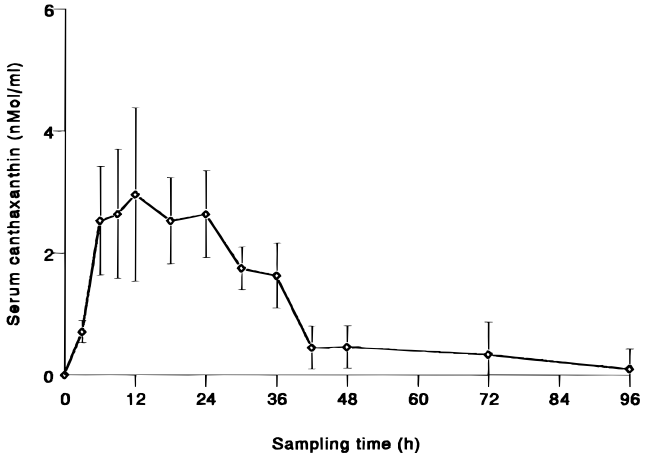

Figure 3. Serum canthaxanthin concentration in rainbow trout following a test meal containing 100 μg of canthaxanthin in the form of 5% water-soluble beadlets. Values are expressed as means of 10 independent determinations ± SEM.

The net change in mean peak serum canthaxanthin concentrations in rainbow trout fed canthaxanthin in the water-soluble beadlet form reached 2.9 ± 0.5 nmol/mL of serum at an average of 12 h post-dosing (Figure 3). A sharp decrease in serum canthaxanthin concentrations occurred from 12 to 42 h, at which time serum concentrations leveled off. By 42 h post-dosing, serum canthaxanthin concentrations had returned to near the baseline (0−0.5 nmol/mL). The individual variabilities were lower than those observed for astaxanthin fed fish.

The comparison of the two curves revealed that the maximum level in the serum of canthaxanthin was 1.6 lower ($p < 0.05$) than that of astaxanthin. However, although there was no significant difference between the level of canthaxanthin and astaxanthin serum concentrations at 3 and 6 h post-dosing, the levels of canthaxanthin serum concentration were always lower than that of astaxanthin from 9 until 72 h.

The disposition of keto-carotenoids from serum in rainbow trout following a single-dose oral administration (100 μg) was best described by a one-compartment model with first-order absorption and a time lag be-

Table 1. Pharmacokinetic Parameters for Astaxanthin and Canthaxanthin Derived from the Mean Serum Concentration Time Data of 10 Rainbow Trout following a Single Oral Administration

parameters[a]	unit	astaxanthin	canthaxanthin
dose	μg	100	100
$(FD/V)\{k_a/(k_a - K)\}$	μg/mL	3.2	3.4
T_{lag}	h	1.09	2.05
rate constant			
absorption (k_a)	h^{-1}	0.1409	0.1382
elimination (K)	h^{-1}	0.0215	0.0612
half-life			
absorption ($T_{1/2}\ k_a$)	h	3.75	5.01
elimination ($T_{1/2}\ K$)	h	34.80	12.63
C_{max}(obsd)	μg/mL	2.6	1.6
C_{max}(calcd)	μg/mL	2.4	1.8
T_{max}(obsd)	h	18	12
T_{max}(calcd)	h	15.9	12.6
AUC	(μg·h)/mL	157.7	52.9
MRT	h	56.5	23.1

[a] $(FD/V)\{k_a/(k_a - K)\} = F$ is the fraction of the dose, D, that is absorbed into the systemic circulation, V is the distribution volume, k_a is the apparent first-order absorption rate constant for carotenoids, and K is the first-order rate constant for elimination of carotenoids by all routes; T_{lag}, lag time; C_{max}, maximum carotenoid concentration; T_{max}, time of the maximum carotenoid concentration; AUC, area under serum concentration−time curve; MRT, mean residence time.

tween oral administration and the apparent onset of absorption:

$$C_t = (FD/V)\{k_a/(k_a - K)\}[\exp\{-K(t - t_{lag})\} - \exp\{-k_a(t - t_{lag})\}]$$

where C_t is the serum concentration, F is the fraction of the dose, D, that is absorbed into the systemic circulation, V is the distribution volume, k_a is the apparent first-order absorption rate constant for carotenoids, K is the first-order rate constant for elimination of carotenoids by all routes, and t_{lag} is the lag time.

Pharmacokinetic parameters (Table 1) describing the disposition of a single oral dose of keto-carotenoids were calculated upon the basis of the above equation. Observed and calculated maximum astaxanthin concentrations in the serum were 38.5% and 25%, respectively, higher than those of canthaxanthin. Observed and calculated times of the maximum astaxanthin concentrations in the serum were 33.3% and 20.7%, respectively, higher than those of canthaxanthin. The calculated half-lives for the absorption phase of astaxanthin and canthaxanthin were 3.7 and 5.0 h, respectively. This means, in terms of absorption speed, that astaxanthin and canthaxanthin are likely equivalent. The relative bioavailability of canthaxanthin with respect to astaxanthin taken as the reference and calculated from the AUC for canthaxanthin (canthaxanthin AUC) over the AUC for astaxanthin (astaxanthin AUC) was 0.335, which corresponds to a percent absorption of canthaxanthin of 33.5% compared to that of astaxanthin. The calculated half-lives for the terminal clearance of astaxanthin and canthaxanthin were 34.8 and 12.6 h, respectively. This shows that astaxanthin was less eliminated from the serum than canthaxanthin. The mean retention time (MRT) of astaxanthin (56.4 h) was 2.4-fold that of canthaxanthin (23.1 h).

Rainbow trout fed astaxanthin beadlets showed (Table 2) a mean liver concentration quite identical on the sampling period. In the intestine the phenomenon was different. The level reached at 9 h was 10-fold that of the liver, and concentrations decreased rapidly from 6 to 1 nmol/g. By 24 h post-dosing, intestine concentration was still 50% of the maximum intestine value. For canthaxanthin the pattern was not the same since canthaxanthin could not be detected because of the low level of canthaxanthin absorption in the rainbow trout in which canthaxanthin analysis was performed (below the limit of detection, 0.03 nmol/mL).

Table 2. Liver and Intestine Concentration (nmol of carotenoid/g of tissue ± SEM, $n = 5$) in Rainbow Trout Following a Test Meal Containing 100 μg of Astaxanthin or Canthaxanthin in the Form of Water-Soluble Beadlets

	time from dosing (h)			
tissue	9	12	24	36
	Astaxanthin			
liver	0.7 ± 0.5	0.2 ± 0.3	0.2 ± 0.2	0.1 ± 0.2
intestine	6.3 ± 0.7	5.1 ± 2.3	3.2 ± 2.3	1.1 ± 0.1
	Canthaxanthin			
liver	ND[a]	ND	ND	ND
intestine	0.2 ± 0.7	0.3 ± 0.1	ND	ND

[a] ND = not detected (detection limit = 0.03 nmol/g of tissue)

DISCUSSION

Fish serum was used in this experiment since it has been reported that plasma extract showed up to 10% lower carotenoid concentrations as compared with the serum extract (Nierenberg, 1984; Stacewicz-Sapuntzakis et al., 1987).

Some fish dosed did not show a serum response to dietary carotenoids for reasons that have yet to be identified. Similar phenomena (designed as "low-responder") were reported previously in rainbow trout (Choubert et al., 1994), calves (Poor et al., 1992), and humans (Meyer et al., 1985). Large variations in concentrations of carotenoids in the serum of fish between individuals have been noted. These variations are not related to dietary intake of these carotenoids since the oral dose was similar.

We have determined the keto-carotenoid (astaxanthin and canthaxanthin) patterns in the serum in rainbow trout after feeding on an oral dose of these compounds in order to define their disposition. Collection of blood samples at frequent intervals has led to an overview of the phenomenon.

This study shows that carotenoid intake can be assessed by measuring serum concentrations of specific carotenoids. After feeding, the concentration of keto-carotenoid in the serum of different individuals increased substantially, the maximum values occurring in the 18th and 12th hours for astaxanthin and canthaxanthin, respectively. This agrees with previous findings for canthaxanthin in rainbow trout (Choubert et al., 1987) but is longer than the time (12 h) reported for astaxanthin in rainbow trout fed diets containing 500 μg of astaxanthin (March et al., 1990). The different astaxanthin preparation used may explain this discrepancy, since in rats the peak serum appearance time was dependent on the formulation (Taylor et al., 1981). But other factors should be taken into account such as environmental factors (temperature or water pH) and factors linked to the physiological state of fish. In contrast, the maximum values in mammals for canthaxathin is reached in only 8 h in calves (Bierer et al., 1995) and 6 h in humans (White et al., 1994) after the meal.

Peak serum astaxanthin concentration in the current work was 1.34-fold lower than the level reported in

rainbow trout fed a diet containing 500 μg of astaxanthin (March et al., 1990). The different astaxanthin preparation may explain this discrepancy since it has been reported that lipids may enhance the absorption of astaxanthin [reviewed by Torrissen et al. (1989)].

In the present study, canthaxanthin, a less polar carotenoid than astaxanthin, had an earlier peak serum appearance time. In the same way in humans, the β-carotene peak serum appearance time was earlier than that of canthaxanthin (White et al., 1994). This suggests that peak serum appearance times seem to be influenced by the polarity of the carotenoid fed: the less polar carotenoid has an earlier peak serum appearance time. However, in calves, results were in the opposite direction (Bierer et al., 1995). On the other hand, canthaxanthin was cleared faster from the serum of trout than astaxanthin. This appears to contradict previous results (Guillou et al., 1992; Choubert et al., 1994) indicating a similarity in the rate of which astaxanthin and canthaxanthin are utilized by rainbow trout. In contrast, in humans (Meyer et al., 1985) and in calves (Bierer et al., 1995), the more polar carotenoids seem to be cleared faster from the serum.

The area under the carotenoid concentration versus time curve describes how much carotenoid is absorbed (as a measure of carotenoid absorption efficiency). The lower absorption rate of canthaxanthin produced a flatter profile and a lower value for the peak serum concentration. Of interest is the relative absorption efficiency of canthaxanthin compared to those of astaxanthin. The ratio AUC canthaxanthin/AUC astaxanthin was 33.5%. This result confirms that astaxanthin is better absorbed than canthaxanthin by rainbow trout [reviewed by Torrissen et al. (1989)]. However, this comparison provides no information regarding the absolute absorption efficiency from either compound. Nevertheless, incomplete absorption of keto-carotenoids has been reported in rainbow trout. Digestibilities ranked from 40 to 60% for astaxanthin (Foss et al., 1987) and from 19 to 30% for canthaxanthin (Choubert and Luquet, 1979) depending on the formulation.

With carotenoid beadlets (enteric-coated dosage form), a finite time period elapsed between the time of carotenoid administration and the time that measurable carotenoid initially appears in serum. This lag time was difficult to detect since the first measure was at 3 h after the meal, but it has been calculated from the one-compartment model equation as 1.09 h for astaxanthin and 2.05 h for canthaxanthin. It is possible that the nature of the coating may explain this lag time since rainbow trout are unable to digest complex starch (Bergot, 1981). Another explanation would be the hydrophilic character of astaxanthin.

The present study reveals a difference in the ability of fish to accumulate keto-carotenoids in the liver. In trout liver astaxanthin was preferentially accumulated over canthaxanthin. The reason for this difference in hepatic accumulation is unknown. It is conceivable that the difference in carotenoids absorption might be due to the ability of animals to form a suitable micellar solution in the intestinal lumen with one carotenoid but not with the other (Bauernfeind et al., 1981). Similarly, due to the difference in the structure of these two carotenoids, it might be that astaxanthin is more suitable for incorporation into the fish lipid micelles.

From a temporal change in serum keto-carotenoid concentrations in rainbow trout, various simulations in fish feeding protocols have been made. One of them (fish receiving alternatively a diet supplemented with keto-carotenoid and unsupplemented) would suggest that a supplemented diet with keto-carotenoids given every other day would give a result similar to that of the same diet given every day, resulting in a saving of carotenoids. Such a suggestion has been made for salmon, *Salmo salar* L., fed a diet supplemented with astaxanthin (Kiessling et al., 1995), but this is a complex matter that requires further investigation.

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