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Pharmacokinetics and Metabolized Caroteniods in Liver of Single Dose Administration in Fancy Carp (*Cyprinus carpio*)

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1. Introduction

The market values of ornamental species are dictated by the qualities of their skin pigmentation, body shape, fin shape and body size (Paripatananont *et al*., 1999). Among ornamental fish, 'fancy carp' (*Cyprinus carpio*) is being popular worldwide due to the increasing number of hobbyists. One of the frequently found problems includes the color of fancy carp which often turns pale after being raised for a period of time, the fact not preferred by hobbyists. In order to make the fancy carp color remain constantly vivid, certain sort of diet, such as 'carotenoids' is believed to help. However, carotenoids cannot be synthesized by most animals, including fish, and must be obtained from dietary sources (Goodwin, 1984). There has been suggestion to modify alimentary carotenoids and store them in the integument and other tissues.

Once digested, carotenoids are absorbed into the plasma, bound to serum lipoproteins (Ando *et al*., 1986) and subsequently transported and distributed to various tissues and organs. The liver is the main metabolic and excretory organ for carotenoids (Torrissen and Ingebrigtsen, 1992) and is considered to have the major responsibility for the metabolic of carotenoids. The liver is therefore either catabolizing astaxanthin to other pigments or to metabolites of carotenoids. The exact process of carotenoids metabolism in the liver is unknown. While the available evidence points to the role of the liver in carotenoid metabolism, no studies have attempted to elucidate its role or to quantify its potential in carotenoid clearance.

Once established, pharmacokinetic studies on the given compound, i.e. the fraction of the feeding dose that is absorbed into the circulation, will be an important determinant of its efficiency and safety (Evan, 2004). In this case, this chapter deals with the quantitative aspects of carotenoid uptake, elimination and metabolic of carotenoid in fancy carps. Pharmacokinetics provides a mathematical basis to assess the time course of carotenoids and their effects in the body. It enables the following processes to be quantified:

- **A**bsorption
- **D**istribution
- **M**etabolism
- **E**xcretion

With this background, this chapter presents the reported evidence for the potential role of the liver in carotenoid utilization and investigation outcomes of the pharmacokinetics of the carotenoids in order to determine the absorption and disposition levels of carotenoids that can partially be ascribed to hepatic first-pass of fancy carps.

2. Carotenoids

Carotenoids can be found in plants and animals. These color-generating materials are in plastids that are naturally occurring in chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria. Though animals cannot produce carotenoids by themselves, they can keep carotenoids as polyunsaturated C40 hydrocarbons which consist of carbon atoms, single bonds along with double bonds. Carotenoids are dissolved in fat called lipophore. Carotenoids are tetraterpenes containing 4 connected isoprenes forming the ring structure. They come in yellow, orange, and red. Light absorption is at 400-600 nm. There are over 600 known carotenoids.

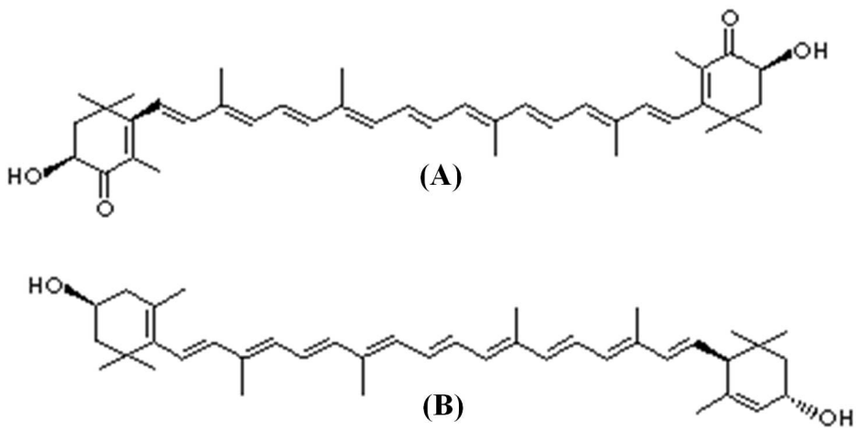


Fig. 1. Chemical structure of carotenoids; A: astaxanthin and B: lutein

Their colors, ranging from pale yellow through bright orange to deep red, is directly linked to their structure. Xanthophylls are often yellow, hence their class name. The double carbon-carbon bonds interact with each other in a process called conjugation, which allows electrons in the molecule to move freely across these areas of the molecule. As the number of double bonds increases, electrons associated with conjugated systems have more room to move, and require less energy to change states. This causes the range of energies of light absorbed by the molecule to decrease. As more frequencies of light are absorbed from the short end of the visible spectrum, the compounds acquire an increasingly red appearance.

3. Digestion and absorption

Carotenoids are lipid soluble and follow the same absorptive path ways as other dietary lipids. This property has been used as a basis for the carotene absorption test, a screening test for lipid malabsorption. Although efficient digestion and absorption of dietary lipid is a prerequisite for optimum absorption of carotenoids, it is suggested that carotenoids are simply absorbed by dietary lipid.

Carotenoid absorption involves several steps from the breakdown of the food matrix and release of carotenoids into the lumen of the gastrointestinal tract through their incorporation into lymphatic lipoproteins. This includes mechanical and chemical disruption of the food matrix, dispersion in lipid emulsion particles, solubilization into mixed bile salt micelles, movement across the unstirred water layer adjacent to the microvilli, uptake by the enterocyte, and incorporation into lymphatic lipoproteins. A perturbation at any point along this chain of events will alter carotenoid bioavailability (Furr and Clark, 1997).

Before absorption, carotenoids must be released from the food matrix, as they are not free in food but are associated with protein in a variety of plant cell structures (Erdman *et al*., 1993). Once the food is ingested, its mechanical breakdown continues as it is chewed, swallowed, and mixed in the stomach. Gastric hydrolysis of dietary lipids and proteins results in partial release of carotenoids and lipids from the food matrix. The extent of release and the physical-chemical state of the carotenoids in the stomach is not known. Once they are released, however, the lipophilic carotenoids would dissolve in an oily phase of lipid droplets. With mixing, the lipid droplets in the gastric contents become emulsified particles. Thus bile salt and fats are required upon carotenoids absorption process (Simpson and Chichester, 1981).

Shearing forces from normal digestive tract motility bring about the formation of a fine lipid emulsion as the contents of the stomach pass into the duodenum. The emulsion has a triacylglycerol core surrounded by a monomolecular layer of partially digested proteins, polysaccharides and lipids, especially phospholipid and partially ionized fatty acids. The solubility and location of the polar carotenoids (xanthophylls) and the nonpolar carotenoids (carotenes) in emulsions differ. Carotenes are thought to incorporate almost exclusively in the triacylglycerol core of the emulsion, whereas the more polar xanthophylls distribute preferentially at the emulsion surface (Borel *et al.*, 1996). Other lipid soluble nutrients with polar groups such as tocopherol and trans-retinoic acid are also thought to locate at the droplet surface. The significance of location in an emulsion is that the surface components can spontaneously transfer from lipid droplets to mixed micelles, whereas components associated with the emulsion core require digestion of triacylglycerol before transfer (Borel *et al*., 1996). The enzyme best suited to hydrolyze triacylglycerol in emulsions is pancreatic colipase-dependent lipase which is one reason why pancreatic insufficiency decreases plasma carotenoid concentrations (Leo *et al*., 1995).

The products of lipid digestion and minor dietary lipids, including the carotenoids, transfer from the emulsion particle to mixed bile salt micelles. Whereas the mechanism of carotenoid solubilization into mixed micelles is unclear, the presence of bile salt micelles is obligatory, as carotenoid absorption is minimal or nonexistent when intraluminal bile salts are below the concentration required for aggregation into micelles (Hollander and Ruble, 1978). A major difference between absorption of other dietary lipid and carotenoids is that the carotenoids seem to have an absolute requirement for bile salt micelles, whereas fatty acids, the major product of lipid digestion, can be absorbed in the absence of micelles.

The solubility of carotenoids in mixed micelles is limited and varies with intraluminal concentration of the carotenoid. Canfield *et a1.,* (1990) studied the incorporation of β-carotene into mixed micelles designed to resemble those seen in the lumen of the small intestine. The incorporation of β-carotene into the micelles varied from approximately 4 to

13% with the percent incorporated decreasing with increasing initial concentration of carotenoid. Whereas the solubility of carotenoids differs in emulsions, the polar and nonpolar carotenoids have similar solubility in bile salt micelles (Borel *et al*., 1996).

Accumulating of carotenoids in fish could be affected from various factors. Apart from the mentioned external and internal ones; digestibility, absorption, blood flowing by lipoprotein and absorbing of carotenoids in muscle fiber can affect digestibility. Apparent digestibility coefficient (ADC) of astaxanthin in rainbow trout shows that the percentage of ADC is at 70% which is higher than 35-70% of canthaxanthin. Differences of ADC and pigmenting efficiency do not depend only at the type of carotenoid, but also at the geometic isomer of each carotenoids themselves.

Addition of fat in the fodder causes enhanced color change in atlantic salmon (Einen and Skrede, 1998). This goes along with a research study conducted by Choubert *et al.* (1991) which reports that adding more fat results in improvement of carotenoids digestion and retention efficiency in rainbow trout (Nickell and Bromage, 1998). It can be concluded that using fat affects pigmentation regimes. The area where high absorption of carotenoids is found lies in posterior intestine. Absorption ability depends on variation in feed intake and is varied upon different types of fish whose triacyglycerols are packaged in chylomicron in enterocytes and pumped throughout the body by metabolized-like chylomicrons. They associate with lipoprotein which acts as the limiting factor. Superfluous carotenoids were discarded without affecting accumulated ones. However, digestibility depends on types of carotenoids. It is found that astaxanthin is well digested in the form of esterified compared to free form.

Polar carotenoids (xanthophylls) are around emulsion while non-polar carotenoids (carotenes) are found in emulsion. Emulsion, however, consists of triacylglucerol core where monomolecular layer e.g. digested protein, plysaccharides and lipids, particularly phospholipids are found. Specifically in ionized fatty acid, polar carotenoids, carotenoids absorption are highly improved compared to non-polar carotenoids. Such absorption is passive diffusion by concentration gradient. It is also found that adding more fat results in better absorption. Carotenoids found in blood circulation are delivered through lipoprotein. Normally, 75% of plasma carotenoids are delivered via LDL and the rest via VLDL and HDL. Non-polar carotnoids such as α, β-carotene, lycopene are delivered via LDL whilst polar carotenoids such as lutein, astaxanthin are delivered via HDL and LDL (Babin and Vernier, 1989).

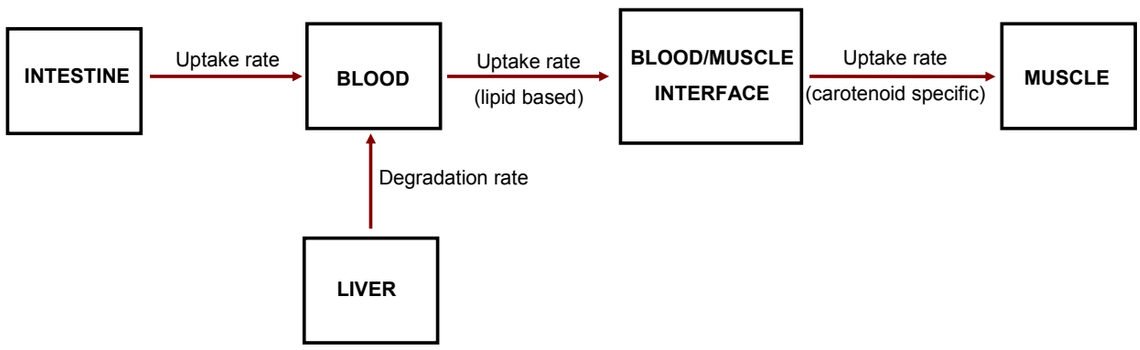
4. Distribution of carotenoids among plasma lipoproteins

Carotenoid content and relation concentrations in human blood, the hydrocarbon carotenes (for example, α, β-carotene and lycopenes) are transported primarily on LDL plus VLDL, whereas the xanthohylls (lutein, zeaxanthin, β-cryptoxanthin) are distributed approximately equally between HDL and LDL in human serum (Johnson and Russell, 1992). Parker (1996) suggested that the actual content of β-carotene per unit lipid (triacylglycerol plus cholesterol) may be greater in HDL than in LDL. Parker also noted that although the total surface area of LDL is approximately twice that of HDL in human plasma, the content of xanthophylls (lutein plus zeaxanthin) is greater in HDL than in LDL.

5. Uptake and transport into blood

Being fat-soluble, dietary astaxanthin is assumed to be in micellar form in the intestine, together with bile salts, fatty acids, monoglycerides and other fat-soluble vitamins. It is believed to passively diffuse into the intestinal lumen, together with fatty acids, and the uptake seems to be a slow process taking between 18 and 30 hours (Choubert *et al*., 1994). The fatty acids are converted into triacylglycerols (TAG), and the astaxanthin, like other xanthophylls (Zaripheh and Erdman, 2002), is incorporated together with TAGs in lipoprotein spheres called chylomicrons. These are then transported into the blood and due to its polarity, astaxanthin is assumed to be attached to the surface of the chylomicron spheres.

In mammals, transport of chylomicrons from the intestinal lumen into the blood is carried out through lymphatic vessels. While being transported along the blood stream, chylomicrons undergo hydrolysis by lipoprotein lipase (LPL), a triacylglycerol lipase found on the surface of endothelial cells of the tissue capillaries, to yield free fatty acids. The fatty acids and monoglycerols that are derived from chylomicrons in this way are subsequently taken up by the tissues or serum albumin. Changes in the lipid composition of a chylomicron modify the affinity of the associated lipoproteins for its surface, causing the chylomicron to change its apolipoprotein signature. When the chylomicrons have lost about 80% of their initial TAG content, they become small enough to pass through the endothelium in the liver, and in addition their apolipoprotein signature can then be recognized by specific receptors in the liver.



Source: Hannah *et al.,* (2006)

Fig. 2. Variation in pigment level due to variation in intestinal uptake likely accounted for by variation in weight to a substantial degree.

The fate of chylomicron-associated xanthophylls is poorly understood in mammals as well as in fish. It is hypothesized that non-triglyceride components of the chylomicron, including surface molecules such as xanthophylls, may be taken up by extra-hepatic tissues or transferred to other blood lipoproteins. When the chylomicrons reach the liver, however, they still contain a considerable amount of their original carotenoid content. Since fish do not seem to have a lymph system similar to the mammalian one, the chylomicrons are assumed to be transported through the primary blood vessels in the intestine. Aside from this, there is reason to assume that chylomicron-based astaxanthin transport and delivery in salmon are quite similar to that in mammals, as discussed above. Studies in salmon have provided evidence that astaxanthin is strongly associated with a protein likely to be serum albumin. Albumin is the major transporter of free fatty acids released during lipolysis by LPL to tissues (including

liver and muscle). This suggests that albumin may acquire astaxanthin from chylomicrons during lipolysis as well as directly from chylomicrons in the bloodstream.

6. Liver metabolism and excretion

The liver is the main metabolic and excretory organ for Carotenoids (Torrissen and Ingebrigtsen, 1992) and is considered to have the major responsibility for the metabolic loss of astaxanthin. The liver secretes bile into the intestine to aid in lipid digestion as well as in the excretion of metabolic breakdown products, and radioactive labelling studies with canthaxanthin found bile radioactivity levels to be 8 times higher than the level in blood. The astaxanthin metabolites in the bile are secreted into the intestine and re-absorbed. Radiolabeling experiments also indicate that either astaxanthin or its metabolites are excreted by the kidneys of salmon and rainbow trout (Hardy *et al*., 1990). The liver is therefore either catabolizing astaxanthin to other pigments or to metabolites that no longer have a chromophore. The exact process of astaxanthin metabolism in the liver is unknown, as is the case with β-carotene metabolism.

7. Transport and deposition in muscle

Despite numerous studies, the mechanism by which free fatty acids enter cells remains poorly understood. Astaxanthin association with LDLs has been observed in salmon (Aas *et al*., 2000) and rainbow trout (Chapman *et al*., 1978), suggesting that astaxanthin-containing LDLs may contribute substantially to the LPL-mediated uptake of astaxanthin by circulating albumin. Astaxanthin is then assumed to be brought to the muscle by circulating albumin. Binding to the muscle cell wall is thought to be non-specific and saturable. After having entered the muscle cell, astaxanthin is deposited in the myotome and binds to actomyosin by weak hydrophobic bonds, forming a complex. The presence of hydroxyl and keto groups at the β-end of the carotenoid increases the binding strength, explaining the higher deposition of astaxanthin compared to other carotenoids in salmon. Metabolites of astaxanthin have also been found in the connective tissues between myotomes.

During VLDL and LDL flow through the blood, some of the TAG found in these lipoproteins is transferred to high density lipoprotein packets (HDL) by the cholesteryl ester transfer protein (CETP) (Tyssandier *et al*., 2002). Given that astaxanthin is transported along with the TAGs to the HDLs, this mechanism is likely to explain the observed high levels of HDL associated astaxanthin in immature salmon (Aas *et al*., 2000), where there is no pigment transport out of the muscle. An interesting feature here is that unlike in mammalian systems, Atlantic salmon muscle has been found to express albumin. Carotenoids are highly accumulated around adipose tissue and liver which are considered significant organs to accumulate carotenoids. The level of carotenoids in each organ directly affects the intake.

8. Pharmacokinetics and metabolized caroteniods in liver of single dose administration in fancy carp (*Cyprinus carpio*)

8.1 Fish and feed trial

Mixed sexes of fancy carp with average weight of 26.93 ± 5.14 g/fish were maintained on a non-pigmented diet for a co-variant period of two weeks prior to feeding the experimental diets, tested in four replicates. Each treatment was randomly distributed to each of 20-liter

aquarium tanks. The diets were designed to achieve a target level of 200 µg for astaxanthin and lutein per fish.

Astaxanthin was supplied at a finished product concentration of 10% astaxanthin (BASF, Thailand). Lutein from marigolds extract (*Tagetes* spp.) was supplied in a finished product containing 15000 ppm (Kemin Industries, Thailand). After administration of a single dose orally, fish were not fed further meals.

8.2 Sampling procedure

Fish were not fed for 3 days before receiving a single dose feeding. A control liver sample at 0 hr, was taken to measure basal liver astaxanthin and lutein levels prior to experiment. Liver sampling occurred at 15, 30 min 1, 3, 6, 12, 24, 48, 72, 96 and 120 hr after single dose meal, three fish were sampled at each sampling time. Liver was immediately separated from blood and stored at -20° C until samples analysis was performed.

8.3 Carotenoids determination

All experimental diets were extracted with acetone, together with BHT (250 ppm) added as antioxidant, until samples showed no color. After that, petroleum ether was added at 5 ml. and mixed, and water was added in a separating funnel. Mixing was carried out with a careful swirling and the two phases were found separated. In this study, only the hyperphase of diets was collected.

8.4 Liver distribution of carotenoids after its oral administration in fancy carp

Approximately 1 g of each liver was quickly excised after sampling (n 3, each). Each liver sample was rinsed with cold 0.9% NaCl solution, blotted dry with tissue paper and weighed. Then each liver was homogenized with acetone, together with BHT (250 ppm) added as antioxidant, until samples showed no color and saponified by addition of 0.2 ml of 60% KOH, followed by 1 hr incubation at 70°C. The saponified mixture was extracted 3 times with petroleum (containing 250 ppm of BHT. In this study, only the hyperphase was collected then determined maximum absorbance wavelength range over 350-600 nm. Merely the maximum absorbance value (λ max) was recorded and calculated total carotenoids were calculated based on Beer-Lambert's Law (Britton, 1995).

8.5 Instrumentation and chromatographic conditions

The resulting hyperphase of experimental diets and liver from petroleum ether extraction were evaporated under a gentle stream of nitrogen gas. This was followed by redissolving crude carotenoids in petroleum ether and the solution 5 µl were spotted on pre-coated silica gel 60 (10 x 20 cm) with 0.25 mm thickness (Merck, Germany) using a CAMAG Linomat IV (Switzerland) sample applicator. A constant application rate of 4 ul/s was employed and spaces between two spots were 14 mm. The slit dimension was kept at 5 mm x 0.45 mm and 20 mm/s scanning speed was employed. The mobile phase consisted of petroleum ether - diethyl ether - acetone (75:15:10, *v/v/v*). Linear ascending development was carried out in a twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ± 2° C). The length of chromatogram run was 70 mm. Densitometric scanning was performed on CAMAG TLC scanner III in the

absorbance mode at 450 nm. The source of radiation utilized was a tungsten lamp (compiled from Mantiri *et al*., 1996 and Sherma and Bernard, 2003). In order to identify the correct value of astaxanthin, lutein and β-carotene, TLC runs were always conducted including authentic standards for astaxanthin and β-carotene (Sigma); lutein (Chromadex, Canada).

8.6 Pharmacokinetic parameter analysis

The astaxanthin and lutein concentration-time curves from liver best fitted one-compartment pharmacokinetic model. The mean hepatic astaxanthin and lutein concentration-time curves data points of three from each time were then analyzed using pharmacokinetic equation (Evans, 2004).

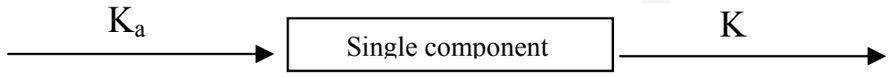


Fig. 3. One-compartment model K_a = absorption rate constant (h^{-1}), k = elimination rate constant (h^{-1})

9. Results

9.1 Pharmacokinetic parameters of fancy carp fed with deprived diets

The astaxanthin and lutein concentration in fancy carp were best described by one compartment model with first order absorption, pharmacokinetic parameters displayed in Table 1. The results showed that hepatic astaxanthin and lutein observed concentration time curve showed a steady rise after post-dosing, a slow increase to the maximum peak at 6 and 24 hr for astaxanthin and lutein, respectively. Astaxanthin and lutein were on a gradual decline after post-dosing.

Parameter	Unit	Parent diets	
		Astaxanthin	Lutein
Vd (area)/kg	ml/kg	1958.80	237.20
CL (area)/kg	ml/hr/kg	8.92	3.50
AUC (area)	µg-hr/ml	5300.00	2082.90
C max (obs)	µg/g	54.20	78.40
T max (obs)	hr	6.0	24.0

Vd (area)/kg: volume of distribution calculations
CL (area)/kg: clearance calculations
AUC (area): area under the serum concentration time curve
C max (obs): maximum observed serum concentrations
T max: observed time at which C max was achieved

Table 1. Pharmacokinetic parameters for astaxanthin and lutein derived from hepatic concentration – time of fancy carp.

Fancy carp fed with an astaxanthin diet developed a maximum concentration of astaxanthin (C max) of 54.20 µg/g occurred at 6 hr (T max) while fancy carp fed with a lutein diet contained lutein hepatic level (C max) of 78.40 µg/g within 24 hr. The volume of distribution (Vd) was 1958.80 and 237.20 ml/kg, respectively. The data illustrated that free astaxanthin was

distributed in the liver better than lutein because the high amount of Vd demonstrates the fact that it can disseminate well in liver. However, the area under the curve (AUC) was 5300.00 and 2082.90 µg-hr/ml. AUC, indicating the relationship between hepatic carotenoid concentration and time. It is close to the amount of carotenoids being absorbed in the liver. If the area under the curve (AUC) is elevated, it means that carotenoids can be highly absorbed. Total body clearance (CL) was 8.92 and 3.50 ml/hr/kg, indicating that the clearance of astaxanthin was greater than lutein. This clearance was mainly to eradicate carotenoids. Overall, after fancy carp were fed an astaxanthin diet, they showed better astaxanthin absorption than lutein. When considered together with pharmacokinetic parameter which was Vd and AUC, well dispersion of astaxanthin in liver has been proved.

The results of the present study indicated that hepatic metabolism of astaxanthin and lutein in fancy carp following oral administration single dose feeding shows the faster rate of astaxanthin absorption for the fish fed with an astaxanthin diet compared to lutein diet which was similar to the report of Olsen and Bakker (2006). The absorption of carotenoids depends on disruption of food matrix. These solubility and location of the polar carotenoids (xanthophylls; astaxanthin and lutein) and the non polar carotenoids (carotene) in emulsions are different. Carotenes are thought to incorporate almost exclusively in the triacylglycerol core of the emulsion, whereas the more polar xanthophylls distribute preferentially at the emulsion surface (Furr and Clark, 1997). It has been shown by several authors, including Yonekura and Nagao (2007) that polar carotenoids (xanthophylls) have higher bioavailability characteristics leading to more transfer across the intestine compared to carotenes. This is related to the polar –OH side chains they possess which are absent in carotenes, showing the absorption of β-carotene appeared to be very low compared to astaxanthin and lutein. In this respect, the transfer rate of astaxanthin would be expected to be slightly higher than that of lutein. They also showed an apparent lack of interaction between astaxanthin and lutein on transfer between emulsions and micelles.

9.2 Metabolized serum carotenoids after administration of single dose of carotenoids diets

Analysis of carotenoids distributed to and metabolized in liver and carotenoids derivatives are shown in Table 2.

Parameters	Unit	Lutein metabolited from Astaxanthin diet	Astaxanthin metabolited from Lutein diet
Vd (area)/kg	ml/kg	4349.40	810.40
CL (area)/kg	ml/hr/kg	35.94	1.27
AUC (area)	µg-hr/ml	516.8	14631.10
C max (obs)	µg/g	65.00	40.9
T max (obs)	hr	12.00	24.00

Vd (area)/kg: volume of distribution calculations
L (area)/kg: clearance calculations
AUC (area): area under the serum concentration time curve
C max (obs): maximum observed serum concentrations
T max: observed time at which C max was achieved

Table 2. Pharmacokinetic parameters of metabolized of hepatic astaxanthin and lutein parent diets derived from concentration – time data of fancy carp

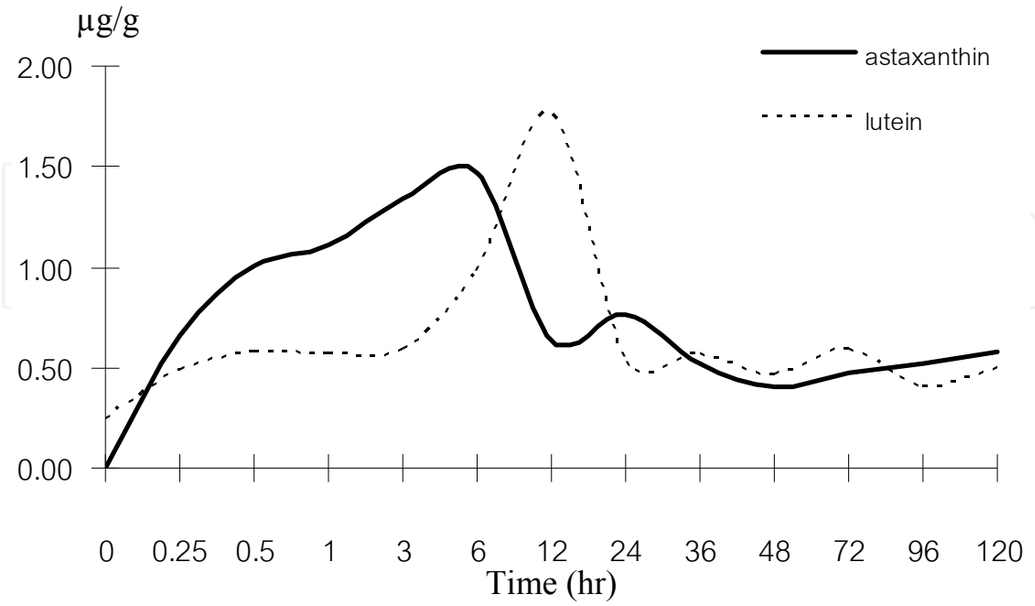


Fig. 4. Hepatic astaxanthin and lutein concentration – time curve for fancy carp administration with a single dose of astaxanthin 200 μg

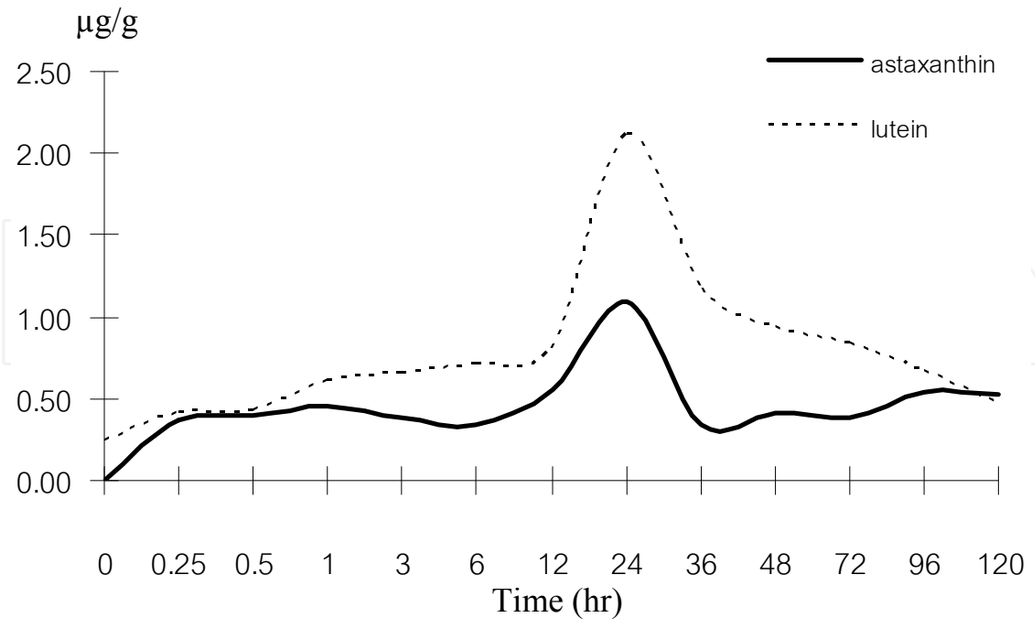


Fig. 5. Hepatic astaxanthin and lutein concentration – time curve for fancy carp administration with a single dose of lutein 200 μg

In this present study, the data showed that the hepatic carotenoids of fancy carp after feeding with an astaxanthin diet were composed of astaxanthin and lutein. Astaxanthin concentration decreased while the time passed, while lutein concentration was increased in liver. In the fancy carp fed with lutein diets, hepatic astaxanthin was found within 24 hr after oral administration feeding. Hepatic astaxanthin concentrations were 40.90 ± 0.03 µg/g. The hepatic astaxanthin concentration continued to increase the serum astaxanthin concentration. It could possibly be assumed that lutein was precursor and converted to astaxanthin, making the level of hepatic astaxanthin concentration higher in liver.

Aquatic animal carotenoids have been reviewed by Goodwin (1984) and Matsuno (2001). Commonly, carotenoids composition in many fishes are β-carotene, β-cryptoxanthin, tunaxanthins, luteins, zeaxanthins, diatoxanthin, alloxanthin, β-echinenone, canthaxanthin, α-doradexanthin, β-doradexanthin and astaxanthins. Carotenoid metabolism in animals takes place as a result of enzymes which catalyse three main types of reaction. These main reaction types are (i) the substitution of carotenoid end groups (often β-end groups) by oxygen functions (-OH and -C=O), (ii) the alteration of end groups, e.g. of β to α and (iii) cleavage of the polyene chain to yield apocarotenoids and even the vitamins A (Davies, 1985).

In this study, after fish were fed with astaxanthin diet, hepatic astaxanthin level was decreased because of metabolic conversions whereas the time hepatic lutein concentration was increased. The results indicated that lutein was carotenoids derived from reductive metabolite of astaxanthin. Based on a minute recovery of radioactivity in lutein after feeding of labeled (3S, 3'S)-astaxanthin, an analogous reductive pathway might also be occurring with gilthead seabeam (Gomes *et al*., 2002). Lutein and zeaxanthin are metabolized prior to β-carotene, suggesting the precedence of xanthophylls biochemical conversions (Berticat *et al*., 2000).

By contrast, after fancy carp were fed with lutein diets, hepatic astaxanthin was found within 24 hr after oral administration. This result suggested that lutein was oxidized to astaxanthin. The data shows that carotenoids were derived in liver after being fed with lutein; and that fancy carp can convert lutein to astaxanthin. The results of this present study were similar to other research studies: gold fish fed with lutein and zeaxanthin diets proposed the possible metabolic pathways from lutein and zeaxanthin to astaxanthin (Ohkubo *et al*., 1999). This indicates that lutein is converted into β-doradexanthin, leading to the formation of astaxanthin by oxidative pathway (Tanaka *et al*., 1976).

The chapter suggesting that absorbed carotenoids in the liver are rapidly metabolized. Torrissen and Ingebrigsten (1992) recovered a high level of radioactivity in the bile of salmon fed 14C astaxanthin, suggesting a role of the liver in pigment metabolism. This is consistent with earlier findings obtained by Schiedt et al. (1988) who reported that astaxanthin represented only 27% of the total carotenoids recovered. Together, the findings suggest that trout may have an efficient hepatic metabolic capacity for the conversion of these carotenoids. Conversely, it appears that salmon are comparatively slower at metabolic conversion of absorbed carotenoids by the liver as evidenced by the fact that the relative hepatic carotenoid level associated with diet was two-fold higher than in trout. Salmon have either a slower hepatic metabolism, a fundamental shift in the tissue responsible for carotenoid catabolism (e.g. kidney or hind gut), or white muscle pigment uptake potential is

lower than in rainbow trout. The liver and kidney appeared to be important sites of carotenoid catabolism based on the relative proportion of the peak chromatogram of the fed carotenoid in rainbow trout and Atlantic salmon. Liver catabolism is suspected to be a critical determinant in carotenoid clearance, with higher catabolism expected in Atlantic salmon than in rainbow trout (Page and Davies, 2006).

In conclusion, this chapter presents the evidence for the potential role of the liver in carotenoid utilization and confirms that the liver plays a significant role in carotenoid metabolism. In addition, the liver appears to be an important organ for the metabolic transformation of carotenoids. Hepatic carotenoids ascribed to first-pass metabolism by pharmacokinetic were designed to investigate dose - response effects on uptake parameters for both astaxanthin and lutein derived from commercial beadlet sources for fancy carp. The first reported evidence for the potential role of the liver in carotenoid utilization showed that liver plays a direct relationship to its potential in metabolism. In addition, the results suggest that the observed discrepancy between absorbed and retained levels of carotenoids can partially be ascribed to first-pass metabolism by the liver, which can be applied in other species research.

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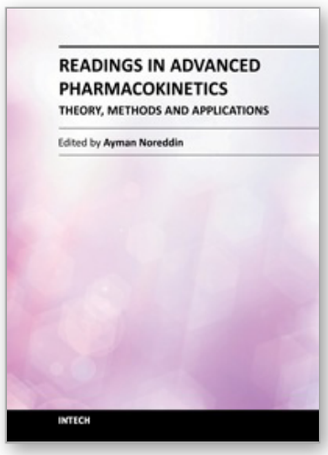

Readings in Advanced Pharmacokinetics - Theory, Methods and Applications

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This book, "Readings in Advanced Pharmacokinetics - Theory, Methods and Applications", covers up to date information and practical topics related to the study of drug pharmacokinetics in humans and in animals. The book is designed to offer scientists, clinicians and researchers a choice to logically build their knowledge in pharmacokinetics from basic concepts to advanced applications. This book is organized into two sections. The first section discusses advanced theories that include a wide range of topics; from bioequivalence studies, pharmacogenomics in relation to pharmacokinetics, computer based simulation concepts to drug interactions of herbal medicines and veterinary pharmacokinetics. The second section advances theory to practice offering several examples of methods and applications in advanced pharmacokinetics.

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