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Digestibility of astaxanthin and canthaxanthin in rainbow trout as affected by dietary concentration, feeding rate and water salinity

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Summary — The digestibility of astaxanthin and canthaxanthin in rainbow trout was studied in two different experiments. In the first experiment, the trout were fed, at a rate of 1.0% body weight/day (BW/d), diets containing various concentrations (12.5, 25, 50, 100 and 200 mg carotenoid/kg feed) of astaxanthin or canthaxanthin in fresh water. The overall apparent digestibility of astaxanthin was higher than that of canthaxanthin whatever the dietary concentration of the carotenoid. Apparent digestibility coefficients were maximum for astaxanthin (79.1 ± 1.3%) at 25 mg/kg feed and for canthaxanthin (68.6 ± 2.8%) at 50 mg/kg feed, and then decreased at higher dietary levels of carotenoids. In the second experiment, only diets containing 25 or 50 mg carotenoid/kg feed of either of the two carotenoids were fed to trout in fresh water at feeding rates of 0.5, 1.0 and 1.5% BW/d, and in sea water (30–32 ppt) at a rate of 1.0% BW/d. Neither the feeding rate nor the salinity affected the digestibility of the two carotenoids.

astaxanthin / canthaxanthin / digestibility / trout / feeding rate / salinity

Résumé — Digestibilité de l'astaxanthine et de la canthaxanthine chez la truite arc-en-ciel. Effet de la concentration alimentaire, du taux de rationnement et de la température de l'eau. *Les digestibilités de l'astaxanthine et de la canthaxanthine chez la truite arc-en-ciel ont été étudiées au cours de deux expériences différentes. Dans la première, des truites, élevées en eau douce, ont reçu, à un taux de rationnement de 1,0 % de leur poids corporel/jour (PV/jour), un aliment contenant différentes concentrations (12,5, 25, 50, 100 ou 200 mg caroténoïde/kilo d'aliment) d'astaxanthine ou de canthaxanthine. D'une façon générale, la digestibilité apparente de l'astaxanthine est plus élevée que celle de la canthaxanthine, quelle que soit la concentration alimentaire. Les digestibilités apparentes atteignent un maximum pour l'astaxanthine (79,1 ± 1,3 %) pour une concentration de 25 mg de pigment/kilo d'aliment et pour la canthaxanthine (68,6 ± 2,8 %) pour une concentration de 50 mg de pigment/kilo d'aliment, puis diminuent pour des concentrations alimentaires en caroténoïdes plus élevées. Dans la seconde expérience, les aliments, contenant 25 ou 50 mg caroténoïde/kilo d'aliment de l'un ou l'autre des deux pigments, ont été distribués à des truites, en eau douce, à des taux de rationnement de 0,5, 1,0 ou 1,5 % PV/jour, et, en eau de mer (30–32 ‰), à un taux de 1,0 % PV/jour. Ni le taux de rationnement ni la salinité de l'eau n'ont affecté la digestibilité des deux caroténoïdes.*

astaxanthine / canthaxanthine / digestibilité / truite / taux de rationnement / salinité

INTRODUCTION

Carotenoids are used in fish culture to enhance the natural pigmentation of the flesh of salmonids, since fish are not able to synthesize these compounds de novo. This is done by supplementing a fish diet with ketocarotenoids (canthaxanthin or astaxanthin) available in the form of gelatin beadlets. However, the digestibility of carotenoids in salmonids shows a large variation ranging from 8–60% for canthaxanthin in rainbow trout, *Oncorhynchus mykiss* (W), fed diets containing 60–200 mg canthaxanthin/kg feed (Choubert and Luquet, 1979; Foss et al, 1987; Torrissen et al, 1990), or 40–90% for astaxanthin in Atlantic salmon, *Salmo salar* (L), or 50–70% in rainbow trout fed diets containing 50–60 mg astaxanthin/kg feed (Foss et al, 1987; No and Storebakken, 1991a). It is unclear, however, if these discrepancies are attribuable to differences in pigment sources, to differences in dietary levels, or to both.

Among the numerous factors which influence the digestibility of diets and their nutrient components, the actual feeding techniques are important (Hasting, 1969). The rate of passage of nutrients through the alimentary tract is a function of digestive balance. Emulsification of the lipid phase, contact time between nutrients and digestive enzymes and the length of time the nutrients remain at the absorptive sites are dependent on the transit rate which is regulated in fish by numerous chemical, physical and biochemical factors, one of which is the feeding rate. In the literature there are conflicting reports on the effect of the feeding level on the apparent digestibility of diets and their nutrients in rainbow trout. For example, the lack of a significant reduction in protein digestibility at increased ration levels was reported by Storebakken and Austreng (1987) while the apparent digestibility of energy decreased with increasing feeding levels (From and Rasmussen, 1984). This factor has not yet been studied for carotenoid digestibility.

Fresh water teleosts do not drink the external medium to maintain their water balance. In contrast, sea water-adapted fish drink seawater to replace body water lost to the hyperosmotic environment by osmosis (Kirsch et al, 1985; Usher et al, 1990). Salinity is reported to affect growth (MacKay and Gjerde, 1985) and body composition of rainbow trout (No and Storebakken, 1991b). Moreover, the digestibility of protein is lower in rainbow trout reared in sea water than in fresh water (MacLeod, 1977; Lall and Bishop, 1979). This is also true for Atlantic salmon (Usher et al, 1990) and Arctic charr, *Salvelinus alpinus* (L), (Ringø, 1991). The digestibility of protein, however, is not affected by salinity in rainbow trout (Brauge et al, 1995). It is, therefore, unclear whether the salinity of the rearing water affects the physiology of trout or the environment in the intestinal lumen. Such an effect on the digestibility of carotenoids has not been studied until now.

With this background, the objectives of the present work were the following: i) to measure the apparent digestibility of ketocarotenoids in rainbow trout given diets containing increasing levels of astaxanthin or canthaxanthin (trial 1); ii) to examine whether increasing the feeding rate affected carotenoid digestibility (trial 2); iii) to ascertain the effect of the salinity of the rearing water on the carotenoid digestibility in rainbow trout (trial 2).

MATERIALS AND METHODS

The experiment consisted of two different trials conducted in France (trial 1) and Norway (trial 2) with the same experimental diets. The digestibility of the carotenoids was determined using chromium oxide as an indigestible marker.

Trial 1

This experiment was carried out at the Hydrobiological Station, Inra, Saint-Pée-sur-Nivelle, France. Twelve groups of 20 rainbow trout, *Oncorhynchus mykiss* (Walbaum), with a mean initial weight of 143 ± 12 g (mean ± SD) were used. Each group was kept in a cylindroconical fibreglass tank (holding capacity: 60 L) with a flow rate of 4 L/min at a water temperature of 10 ± 1 °C and a 12 h photoperiod.

The formulation and the chemical composition of the basal diet is shown in table I. Diets were supplemented with 0.0, 12.5, 25, 50, 100 or 200 mg astaxanthin or canthaxanthin per kg feed. Carotenoids were supplied in the form of dry beadlets containing emulsified carotenoid in nearly colloidal-size oil droplets in a matrix of gelatin (F Hoffmann-La Roche Ltd, Basel, Switzerland). Diets were pelleted through a 4 mm dye and stored in a cool place. Fish were adapted to the tanks for 15 days and were hand fed once daily at 0900 hours with their respective diets at a feeding rate of 1% body weight/day (BW/d). The faeces were continuously collected using the automatic fish faeces collector according to Choubert et al (1982). This apparatus consisted of a cylindroconical fibreglass tank from which the effluent water was drained over a rotating screen filter, separating the faeces immediately (within 14 s) from the water and propelling them to a refrigerated pan. The collected faeces were pooled over two periods of 5 days for each tank, freeze-dried and stored at –18 °C prior to analysis.

Table I. Formulation and chemical composition of the basal diet.

Ingredient	*Amount (% weight)*
Capelin meal	60.0
Extruded wheat	24.0
Soyabean oil	7.0
Cod liver oil [a]	5.0
Vitamin and mineral premix [b]	2.0
Chromium oxide	1.0
Lignin sulfate	1.0
Chemical analysis	
Dry matter	90.5 ± 2.0
Crude fat	17.1 ± 1.2
Crude protein [c]	50.8 ± 0.9
Total energy [d]	1.2 ± 0.2

Dry matter values are percentages of wet weight; other values are percentages of dry weight. [a] Supplemented with etoxyquin (200 mg/kg); [b] Storebakken et al (1987); [c] Kjeldahl's method after acid digestion (%N x 6.25); [d] kJ.g dry matter basis, IKA adiabatic bomb calorimeter.

Trial 2

This experiment was carried out at the Institute of Aquaculture Research Ltd (AKVAFORSK), Sundalsøra, Norway. Twenty groups of 25 rainbow trout with an individual mean initial weight of 111 ± 6 g were used. Each group was kept in a 1 m^2 indoor fibreglass tank supplied with either seawater or fresh water and having a 24 h daylength. The fish were fed, by automatic disc feeders (Åsgård and Nes, 1986), the same basic diet as in trial 1, supplemented with either 25 or 50 mg carotenoid/kg feed. The trout grown in fresh water, at an average temperature of 7.2 °C, were fed at three feeding rates (0.5, 1.0 and 1.5% BW/d). The daily rations were corrected for fish growth and increased twice a week, assuming a feed/gain ratio of 1.0 kg feed/kg growth. The trout in saltwater, with an average temperature of 7.4 °C and a water salinity of 29–32‰, were fed the same basic diet, those supplemented with either 25 or 50 mg carotenoid/kg feed, at a feeding rate of 1.0% BW/d. Faeces were collected by stripping according to Austreng (1978). After the fish were anaesthetized with chlorobutanol, the examiner's left hand held the head, while the forefinger and thumb of the right hand, embracing the body, moved with pressure from the ventral fins to the anus. A faeces column of 0.5–1.5 cm was stripped into a pan. The collected faeces were pooled, freeze-dried, and stored at –18 °C prior to analysis.

Chemical analyses and calculation

Diets and faeces were analysed for dry matter (drying at 105 °C to constant weight), lipids (Folch

et al, 1957), chromium oxide after perchloric acid digestion (Bolin et al, 1952) and carotenoids (Choubert and Storebakken, 1989).

The apparent digestibility coefficient was calculated according to the following relationship (Maynard and Loosli, 1969):

$$ADC = 100 [1 - (CF / CD \times \%CRD / \%CRF)]$$

where ADC is the apparent digestibility coefficient; CF, the μg carotenoid/g faeces; CD, the μg carotenoid/g diet; %CRD, the percentage of chromium in the diet; and %CRF, the percentage of chromium in the faeces.

Apparent digestibility coefficients of carotenoids were subjected to analysis of variance, Duncan's multiple-range test and *t*-test procedures using the GLM procedure (SAS, 1985). Significance was indicated for $P < 0.05$.

RESULTS

Diets

The analysed concentrations of carotenoid in the diets (table II) were higher than the expected values for carotenoid concentrations lower than 50 mg pigment/kg feed and lower for higher carotenoid concentrations.

Table II. Carotenoid concentration of the experimental diet supplemented with astaxanthin (5% formulation) and canthaxanthin (10% formulation).

Carotenoid	*Expected concentration* [1]	*Analysed concentration* [2]
Astaxanthin	12.5	17.7 ± 3.2
	25	27.5 ± 0.9
	50	43.1 ± 2.7
	100	72.0 ± 1.9
	200	174.3 ± 2.3
Canthaxanthin	12.5	14.3 ± 3.8
	25	26.2 ± 1.1
	50	45.5 ± 2.2
	100	70.1 ± 1.3
	200	169.7 ± 3.2

[1] mg carotenoid/kg feed; [2] mg carotenoid/kg feed on a dry weight basis (mean ± SD, $n = 3$).

Trial 1

The overall digestibility of the dry matter and total lipid content showed no significant differences among the experiment conditions (84.2 ± 0.3% and 85.1 ± 0.4% for diets supplemented with astaxanthin and canthaxanthin and 97.3 ± 0.2% and 97.5 ± 0.1%, respectively). The apparent digestibility of astaxanthin was significantly ($P < 0.05$) higher than that of canthaxanthin (table III). The digestibility of carotenoids was affected by dietary concentrations: high doses of keto-carotenoid in the diet decreased the digestibility of carotenoid. The digestibility was maximum for astaxanthin at 25 mg astaxanthin/kg feed and for canthaxanthin at 50 mg canthaxanthin/kg feed.

Trial 2

The digestibility of carotenoids for diets containing 25 mg astaxanthin/kg feed and 50 mg canthaxanthin/kg feed were not affected by the feeding rate (table IV). However, for the two other diets (50 mg astaxanthin/kg feed and 25 mg canthaxanthin/kg feed), the digestibility of carotenoids increased as the feeding rate increased. Canthaxanthin digestibility coefficients were always lower than those of astaxanthin, except for the diet supplemented with 25 mg canthaxanthin/kg feed.

Carotenoid digestibility levels obtained in rainbow trout reared in fresh water tended to be higher than that of sea water-adapted fish (table V). Nevertheless, no significant

Table III. Apparent digestibility coefficients (ADC) of astaxanthin and canthaxanthin in rainbow trout fed various dietary concentrations of carotenoids and reared in fresh water at 17 °C (trial 1).

Dietary carotenoid concentration mg/kg feed	*ADC of astaxanthin (%)*	*ADC of canthaxanthin (%)*
12.5	72.2 ± 3.0 ^{b,*}	54.7 ± 1.0 ^{d,**}
25	79.1 ± 1.3 ^{a,*}	65.6 ± 3.9 ^{ab,**}
50	73.7 ± 2.8 ^{b,*}	68.6 ± 2.8 ^{a,*}
100	64.2 ± 0.4 ^{c,*}	61.4 ± 2.7 ^{bc,*}
200	61.1 ± 3.9 ^{c,*}	58.1 ± 1.4 ^{cd,*}

Means ± SD are given, $n = 4$. ^{abcd} Within a column, values with a common letter(s) are not significantly different (Duncan's multiple range test, $P < 0.05$). *,**Within a row, values with a common asterisk(s) are not significantly different (*t*-test, $P < 0.01$).

effect of salinity on the carotenoid apparent digestibility coefficients was observed.

DISCUSSION

In the aquatic environment, as both feed and faecal particles are transited through the same medium, to separate faeces from water and to avoid contamination of the faeces by uneaten feed, it is necessary to use approaches other than those used to measure digestibilities with mammals or birds (Cho and Kaushik, 1990). The collection of fish faeces has always presented specific problems such as faeces leaching and fish stress. Although faecal collection by strip-

Table IV. Effect of feeding rate on the apparent digestibility coefficients (ADC) of astaxanthin and canthaxanthin in rainbow trout fed two dietary levels of keto-carotenoids and reared in fresh water at 7.2 °C (trial 2).

Feeding rate (% BW/d)	*Astaxanthin ADC Diet supplementation 25 mg/kg feed (%)*	*Astaxanthin ADC Diet supplementation 50 mg/kg feed (%)*	*Canthaxanthin ADC Diet supplementation 25 mg/kg feed (%)*	*Canthaxanthin ADC Diet supplementation 50 mg/kg feed (%)*
0.5	88.7 ± 0.9 ^{a,*}	76.3 ± 3.4 ^{b,**}	76.6 ± 4.4 ^{b,**}	77.7 ± 5.2 ^{a,**}
1.0	89.6 ± 6.6 ^{a,*}	missing	79.6 ± 8.6 ^{b,*,**}	77.0 ± 0.8 ^{a,**}
1.5	87.2 ± 6.9 ^{a,*}	90.8 ± 2.1 ^{a,*}	91.6 ± 1.9 ^{a,*}	72.7 ± 2.7 ^{a,**}

Means ± SD are given, $n = 3$. ^{ab} Within a column, values with a common letter(s) are not significantly different (Duncan's multiple range test, $P < 0.05$). *,** Within a row, values with a common asterisk(s) are not signifiantly different (*t*-test, $P < 0.01$). BW/d: body weight/day.

Table V. Effect of water salinity on the apparent digestibility coefficients (ADC) of astaxanthin and canthaxanthin in rainbow trout fed two dietary levels of keto-carotenoids (trial 2).

	Astaxanthin ADC Diet supplementation 25 mg/kg feed (%)	*Astaxanthin ADC Diet supplementation 50 mg/kg feed (%)*	*Canthaxanthin ADC Diet supplementation 25 mg/kg feed (%)*	*Canthaxanthin ADC Diet supplementation 50 mg/kg feed (%)*
Fresh water	89.6 ± 6.6 [a,*]	missing	79.6 ± 8.6 [a,*]	77.0 ± 0.8 [a,*]
Sea water	73.3 ± 4.5 [a,*,**]	87.9 ± 1.6 *	67.2 ± 2.9 [a,**]	64.2 ± 6.8 [a,**]

Means ± SD are given, $n = 3$. [a] Within a column, values with a common letter are not significantly different (Duncan's multiple range test, $P<0.05$); *,** Within a row, values with a common asterisk(s) are not significantly different (t-test, $P < 0.01$).

ping avoids the problem of leaching, the samples are not fully representative of faeces naturally released, resulting in lower digestibility levels (Spyridakis et al, 1989; Hajen et al, 1993). Use of an automatic fish faeces collector which removes faeces from water within seconds, leaving the fish undisturbed, gives more reliable results (Spyridakis et al, 1989). However, in the present study, digestibility coefficients were abnormally high in trial 2, although the difference was not significant. Three hypotheses have to be taken into consideration in order to explain these results: i) faeces filtered from water were leached resulting in an underestimation of digestibility; ii) the non-uniformity of the digestion process led to variations in nutrient absorption resulting in a nonrepresentative sample; iii) the stripping faeces contained residues or artefacts resulting in an overestimation of digestibility coefficients. Consequently, data from trial 1 and 2 were not compared.

Digestibility of astaxanthin was higher than that of canthaxanthin in rainbow trout. This difference might be ascribed to the fact that the former possess two hydroxy-groups in addition to the two keto-groups in the ring, while the latter is only a keto-hydrocarbon and that esterification of the hydroxy-groups is the first step in the absorptive process (Young and Fox, 1936). However, as the carotenoids used in our experiment were unesterified, the contact time between the carotenoid preparation and the digestive enzyme would be more important with respect to the carrier medium than with the carotenoid itself. Another factor which may be of importance, since carotenoids are lipid soluble, is the presence of mixed micelles representing the form of transport of amphiphates from their site of appearance to their site of uptake in the intestinal lumen (Léger, 1985).

Apparent digestibilities of carotenoids were studied over a large range of dietary concentrations from 12.5 mg/kg feed up to 170 mg/kg feed. The apparent digestibility coefficients of carotenoids showed a maximum for astaxanthin at 25 mg/kg feed and for canthaxanthin at 50 mg/kg feed and then decreased for higher doses of carotenoids in the feed. This pattern is different from the negative linear relationship reported by Torrissen et al (1990) for canthaxanthin in rainbow trout grown in fresh water, who assumed that carotenoids in fish are absorbed by a specific process. Differences

in methodology used (dissection vs faeces collector) may explain these discrepancies. An alternative explanation would be that the lipoprotein fractions that carry carotenoid in the blood have ample binding capacity. However, whether this levelling off was due to an inability to absorb more carotenoid or to a saturation of the lipoprotein binding sites, could not be determined from the present data. A similar conclusion was drawn for β-carotene (Mathews-Roth and Gulbrandsen, 1974).

Our results indicated that the apparent digestibility of carotenoids in fish fed at a wide range of feed intake levels did not differ significantly. The fact that the range of feed intakes used were lower than the satiation amount for fish of this size and at these temperatures can partly explain this result since at higher feeding levels the passage rate of dietary material through the digestive tract has been reported to be higher with the result that less material is digested and absorbed (Elliot, 1976; From and Rasmussen, 1984). Similar findings have been reported for dry matter, crude protein, lipid and energy (Staples and Nomura, 1976; Pedersen, 1987; Cho and Kaushik, 1990). Nevertheless, for the astaxanthin (25 mg/kg) and canthaxanthin (50 mg/kg) supplemented diets, higher feeding levels gave higher digestibility coefficients. A possible explanation, as in trial 2 where the trout were fed the experimental diets continuously, would be the ability of meal-feeding to readily entrain some activity and circulating nutrient rhythms but not another. This was reported for carbohydrates in rainbow trout (Murai et al, 1983) or cortisol and thyroxine (T4) in goldfish, *Carassius auratus* (Spieler and Noeske, 1984).

The effect of salinity on protein digestibility gave contradictory results. The digestibility of dietary protein decreased significantly in rainbow trout (MacLeod, 1977; Lall and Bishop, 1979), in Atlantic salmon (Usher et al, 1990), or in Arctic charr (Ringø, 1991) with an increasing of salinity. On the other hand, salinity had no significant effect on protein digestibility in rainbow trout (Dabrowski et al, 1986; Brauge et al, 1995). In the present study salinity had no significant effect on carotenoid digestibility in rainbow trout despite the fact that digestibility of carotenoids in fish reared in fresh water has a tendency to be higher than those in fish reared in seawater. The reason for the decrease in digestibility with increasing salinity is not known, but it is possible that seawater-rearing affects digestive and absorptive processes of food because of the food motility changes necessitated by osmoregulatory processes (MacLeod, 1977).

CONCLUSION

Results from this present and previous studies (Choubert and Storebakken, 1989) showed that the absorption of carotenoids is maximal for dietary levels up to 25 mg astaxanthin/kg feed and 50 mg canthaxanthin/kg feed, respectively, in rainbow trout. Therefore, high levels of dietary carotenoid supplementation must be avoided. These conclusions, however, were obtained with a well digested diet containing up to 12% oil. Similar studies with diets that are less digestible and/or have a lower oil content would also yield interesting information.

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