



**Opinion of the Scientific Committee on Animal Nutrition
on the use of canthaxanthin in feedingstuffs
for salmon and trout, laying hens, and other poultry**

Adopted on 17 April 2002

1. BACKGROUND

Canthaxanthin has been authorised at Community level under EC number E 161g as a colouring matter in feedingstuffs under the conditions set out in Council Directive 70/524/EEC on additives in feedingstuffs (see table hereafter).

EC N°	Additive	Chemical formula, description	Species or category of animal	Maximum Age	Maximum content mg/kg complete feedingstuff	Other provisions
E 161g	Canthaxanthin	C ₄₀ H ₅₂ O ₂	Poultry	-	80 (alone or with the other carotenoids and xanthophylls)	
			Salmon, trout	-	80	Use permitted from the age of 6 months onwards The mixture of canthaxanthin with astaxanthin is allowed provided that the total concentration of the mixture does not exceed 100 mg/kg in the complete feedingstuff.
			Dogs, cats and ornamental fish	-		
			Pet and ornamental birds	-		

The SCAN has delivered an opinion in December 1982 (fourth series) on the use of canthaxanthin in feedingstuffs for salmon and trout.

The FAO/WHO Joint Expert Committee on Food Additives (JECFA)(1996) assessed the intake of canthaxanthin, a food additive used to colour foods directly through its use in animal feeds. JECFA evaluated at several times canthaxanthin. It established an ADI for canthaxanthin of 0 - 25 mg/kg bw at its 18th meeting, which was subsequently reduced tentatively to 0 - 0.5 mg/kg bw at its 31st meeting. In 1995, at the 44th meeting of JECFA the current ADI was established as 0 – 0.03 mg/kg bw.

The Scientific Committee on Food (SCF) assessed canthaxanthin in 1983, 1989 and 1990. The SCF in 1997 concluded that the lowest effect level for ERGb-wave changes (i.e. parameter for general retinal damage measured by electroretinography) in man was 0.25 mg/kg bw/day but in view of the fact that these changes were not of pathological significance or indicative of significant functional damage to the retina, a safety factor of 10 could be considered appropriate. This was supported by the finding of a one order of magnitude difference between the plasma level (156 µg/L) at the no effect level (NEL) in monkeys and the *in vitro* concentration (1200 µg/L medium) first showing the presence of intracellular microcrystal formation in neuronal retina reaggregate cultures (SCF, 1997).

An ADI of 0.025 mg/kg bw, rounded up to 0.03 mg/kg bw was therefore established by the SCF. However, the SCF considered that up-to-date information should be obtained on human intake from the use of canthaxanthin in animal feeds to give assurance that total exposure by this route would not exceed the ADI. The SCF also noted that reliable intake estimates were not available at present but data suggest that 0.2 mg/egg and 0.1 mg/100 g fish were representative residue levels.

2. TERMS OF REFERENCE

In the light of the revision of the ADI by the Scientific Committee on Food (opinion of June 1997), the Scientific Committee on Animal Nutrition is asked to review the maximum levels of canthaxanthin in feedingstuffs for laying hens, for other poultry, for salmon and for trout which ensure the safety for the consumer, with particular regard to vulnerable groups within populations, consuming foodstuffs containing canthaxanthin.

In making its assessment the Committee is requested to indicate the minimum level of canthaxanthin in the product necessary to observe the technological effect.

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3. INTRODUCTION

3.1. Definition of carotenoids

Carotenoids are isoprenoid polyenes, a chemical group which includes canthaxanthin (chemically described as β,β -carotene-4,4'-dione).

The carotenoids are categorised as follows: (a) vitamin A precursors that do not pigment, such as β -carotene, (b) pigments with partial vitamin A activity, such as cryptoxanthin, β -apo-8'-carotenoic acid ethyl ester, (c) non-vitamin A precursors that do not pigment or pigment poorly, such as violaxanthin and neoxanthin, and (d) non-vitamin A precursors that pigment, such as lutein, zeaxanthin, and canthaxanthin. Because of the numerous conjugated double bonds and the cyclic end groups, carotenoids present a variety of stereoisomers with different chemical and physical properties. The most important forms commonly found among carotenoids are geometric (*E*- / *Z*-). A double bond links the two residual parts of the molecule either in an *E*-configuration with both on opposite sites of the plane, or *Z*-configuration with both on the same side of the plane. Geometrical isomers of this type are interconvertible in solution. This stereoisomerism exerts a marked influence on the physical properties. Isomers differ not only in their melting points, solubility and stability, but also in respect to absorption affinity, colour and colour intensity. No data on isomers of canthaxanthin are known.

3.2. Sources of carotenoids and especially canthaxanthin

3.2.1. Natural carotenoids

Carotenoids are synthesised only by plants and are responsible for many of the brilliant colours in flowers and fruits (Weedon, 1971).

Carotenoids occur in the animal kingdom, especially in insects, birds and fish, but animals depend entirely on their feed for their supply. Although animals cannot synthesise carotenoids *de novo*, some of them can however convert carotenoids into functionally active retinoids (retinol and derivatives).

Birds living in the open and/or fed usual feed ingredients like maize or alfalfa deposit in the fat and transfer to the eggs the carotenoids normally present in these plants.

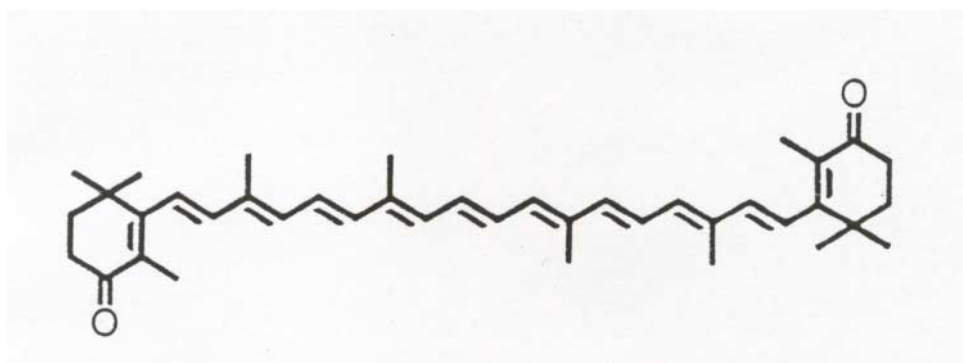
For planktivorous fish, the primary dietary source of carotenoids is phytoplankton, which produces these compounds by primary biosynthetic processes. Secondary sources are marine animals that have accumulated quantities of carotenoids from their consumption of phytoplankton, and which are subsequently consumed by other marine animals that are higher up on the food chain. The nature and concentrations of carotenoids in wild fish tissues vary depending on the geographical and environmental conditions (Gilchrist and Lee, 1972). Main sources relevant to the food chain of wild salmonids are

zooplankton (including calanoid copepods and krill), other crustaceans, and red fish oils (including capelin oil). These natural sources provide essentially astaxanthin.

3.2.2. Natural and synthetic canthaxanthin

Canthaxanthin is the β,β -carotene 4,4'-dione (Figure 1).

Figure 1 Chemical structure of canthaxanthin



Canthaxanthin was first isolated from the edible mushroom, *Cantharellus cinnabarinus* (Haxo, 1950). Canthaxanthin is also stated to be produced in several green algae as secondary carotenoids at the end of the growth phase instead of, or in addition to, primary carotenoids (Czygan, 1968), and as well as in blue-green algae (Hertzberg and Liaaen-Jensen, 1966). It has also been found in bacteria (Saperstein and Starr, 1954), crustacea (Davies *et al.*, 1970; Thommen and Wackernagel, 1964) and various species of fish including carp *Cyprinus carpio* (Katayama *et al.*, 1971; 1973), golden mullet *Mugil auratus*, annular seabream *Diplodus annularis*, and trush wrasse *Crenilabrus tinca* (Czeczuga, 1973). Canthaxanthin is not encountered in wild Atlantic salmon but represents a minor carotenoid in wild Pacific salmon (Kitahara, 1983; 1984a,b; Matsuno *et al.*, 1980). It was also reported in the wild trout *Salmo trutta* (Thommen and Gloor, 1965).

Canthaxanthin was first synthesised from β -carotene (Petracek and Zechmeister, 1956), followed by complete synthesis by Isler *et al.* (1956) and by Isler and Schudel (1963). Canthaxanthin has been produced by chemical synthesis since 1962.

3.3. Use of carotenoids

Colour is an important characteristic and selection criterion for food choice by consumers. Recent studies have highlighted its importance and have shown how preference may change among certain populations and over time (Clydesdale, 1993). With this regard, carotenoids are used to colour food products of animal origin. In order to meet market needs and considering the inherent variations of the diets in terms of colouring agents, the feed industry adds colouring agents to salmonid feed (to colour the flesh), to laying hens

diet (to modify the yolk pigmentation) and, in some areas, to chicken diet (to modify skin pigmentation). Canthaxanthin is one of the carotenoids authorised for use in animal feed as a colouring agent in poultry feeds and fish feeds in accordance with Council Directive 70/524/EEC as E-161g.

Canthaxanthin is also a colour additive for food (E-161). Its use is restricted to the colouring of Strasbourg sausages to a maximum concentration of 15 mg/kg. Canthaxanthin (E-161) is also permitted as a colouring agent for medicinal products.

3.4. Carotenoids and feed technology

Commercially produced feedingstuffs for fish and broilers are usually pelleted or extruded. Both processes involve heat and pressure and that can destroy part of the carotenoids. Further loss can occur upon storage. In contrast to carotenoids of natural origin, those produced by chemical synthesis are characterised by a defined degree of purity and greater stability (Bernhard, 1990). However chemically synthesised carotenoids remain sensitive to oxidation processes (Bartov and Bornstein, 1966). It has been reported that pellets kept for 2 months under ambient temperature lose 20% of their canthaxanthin content (Choubert and Luquet, 1979). For this reason, antioxidants such as ethoxyquin are used in combination (Seemann, 1999; Nys, 2000).

4. USE OF CANTHAXANTHIN IN FISH

4.1. Consumer choice and food technology constraints

For consumers, pigmentation is regarded as the most important criterion after freshness for farmed salmonids (Koteng, 1992). It has traditionally been held that coloration is related to superior flavour, an opinion that still persists (Clydesdale, 1993; Sylvia *et al.*, 1995; 1996). However, there is no standard colour. The salmonids that are marketed have various colours from pale, faintly pigmented fish, to strong red tones.

Market requirements varied over time. The recent trend has been towards increased pigmentation. Between 1976 and 1982, fish flesh contained up to 2.5 mg carotenoids (mainly added canthaxanthin) per kg of flesh in Norwegian farmed Atlantic salmon. Then, between 1982-1988, the concentrations increased up to around 3-5 mg/kg.

Today the concentration of carotenoids (mainly canthaxanthin and astaxanthin) exceeds 8 mg/kg of flesh and all producers try to reach a level that represents a value of 16 on the "Roche Color Card" (Torrissen, 2000). It must be noted that this scale is specific for measuring the pink colour due to astaxanthin and is not adapted to the orange hue obtained with canthaxanthin. Although a linear relationship has been established between the Color Card Score and the astaxanthin content in fish flesh, no such data is available for canthaxanthin

The development over time of processing and storage operations, which can impact on canthaxanthin flesh concentration (see 3.4), has led to an increased quantity of pigments added to the diet to compensate for the degrading effects of processing.

In wild fish, carotenoid levels of up to 20-25 mg/kg have been reported in the flesh of trout (Storebakken and No, 1992), a value comparable to that reported for coho salmon by Schiedt *et al.* (1981). It must be noted that canthaxanthin, when present, is a very minor component (see 3.2.2).

4.2. Practical conditions of fish colouring

4.2.1. Relationship between diet pigmentation and muscle colouring

Fish flesh is toned by red carotenoid pigments from pale to reddish, or, as in salmonid fish, from reddish to pink, but the relationship between visual score and carotenoid level is linear only at low carotenoid levels in fish muscle. At least two factors contribute to this: firstly, the human eye has a limited capacity to distinguish differences in flesh colour when carotenoid concentration exceeds a level of 3-4 (Torrissen *et al.*, 1989) to 6-7 mg carotenoid/kg of flesh (Skrede *et al.*, 1990), and secondly, unpigmented intermuscular fat may mask the impression of colour.

4.2.2. Current practice

Among the species of salmonids farmed worldwide, rainbow trout is widely cultured both for food production and for recreational fishery. When farming is aimed at producing food fish, the trout are reared from egg until they reach table-size (or portion-size) or larger, but in this case fish that are used are often genetically triploids to avoid drawbacks of fish sexual maturation. Triploid and diploid fish show a similar ability to fix canthaxanthin (Choubert and Blanc, 1985). In many countries rainbow trout are harvested when they are portion-sized fish, but in some other countries they are grown to larger size (up to 3 kg) before slaughter. Atlantic salmon are grown to large size (up to 6 kg). Most of the other salmonids - Pacific salmonids (genus *Oncorhynchus*), charr (genus *Salvelinus*) and trout (genus *Salmo* and *Oncorhynchus*) - are raised primarily for stock enhancement and recreational fishery purposes (Jobling, 1993).

Three different pigmentation strategies are currently applied in intensive salmonid farming:

- for portion-size trout, canthaxanthin is added to the complete feedingstuff at a concentration of 80 mg/kg. The feed is then distributed to fish for an average of 6 to 8 weeks before slaughtering.
- for larger fish, canthaxanthin is added at a concentration of up to 80 mg/kg feed, depending on the pigmentation regime, from the weight of 150-200 g and for the whole life of the fish (up to 6 kg).

- some salmonid feeds contain a combination of canthaxanthin and astaxanthin in the diet at a maximum concentration of 100 mg total carotenoids/kg feed.

4.3. Deposition of canthaxanthin in the flesh

4.3.1. Fate of canthaxanthin in fish

In salmonids, the absorption of 4,4'-oxo-carotenoids like canthaxanthin and astaxanthin is more efficient than that of 3,3'-hydroxy-carotenoids (Schiedt *et al.*, 1985). Administration of ³H-labelled canthaxanthin to rainbow trout showed a wide individual variation in the total serum concentration, a peak was attained at 24 hours while most of the radioactivity was found in the pyloric caeca, ovary and skin. After 72 hours most of the radioactivity was recovered from the muscle, liver and kidney (Choubert *et al.*, 1987; Gobantes *et al.*, 1997). Considerable biliary excretion occurs (about 8-fold blood concentration), but unchanged canthaxanthin has not been identified in the bile thus indicating extensive biotransformation of the compound (Hardy *et al.*, 1990).

Schiedt and co-workers (1988) have established that in the skin of Atlantic salmon administered a canthaxanthin-supplemented diet with very low natural carotenoid contents, canthaxanthin and its metabolites represented 14% and 70% of total carotenoids, respectively. Biotransformation pathways have been identified that lead either to the loss of one oxo group giving rise to echinenone, or to the reduction of one oxo group to 4'-hydroxy- β,β -carotene-4-one then of the second oxo group giving rise to β,β -carotene-4,4'-diol (isozeaxanthin), the end product being β,β -carotene. Neither the direct oxidation, nor the epoxidation of intermediary reduced species such as that occurring with zeaxanthin, an astaxanthin reduced metabolite, were observed with canthaxanthin.

A recent metabolic investigation carried out in the rat (Bausch *et al.*, 1999) allowed the identification of a major urinary metabolite, the 3-hydroxy-4-oxo-7,8-dihydro- β -ionone, which is not present in fish, indicating different metabolic pathways.

4.3.2. Distribution of canthaxanthin in salmonids

Canthaxanthin is mainly deposited in the flesh of salmonids due to binding to myofibrillar actomyosin. The retention (defined as the proportion of the ingested canthaxanthin which is retained in the flesh or in the whole body) rate decreases with increasing dietary doses.

Carotenoids are found also in the skin mainly along the lateral line and in specific cells, xanthophores (review by Goodwin, 1984). Immature rainbow trout accumulates approximately 10% of the absorbed carotenoids in the skin (Choubert, 1977).

During sexual maturation considerable amounts of carotenoids (up to 18% of the total body content according to Sivtseva, 1982) are transferred to eggs in wild trout. Similarly in farmed trout the canthaxanthin ingested by the females is transferred to eggs and then to larvae (Choubert *et al.*, 1998).

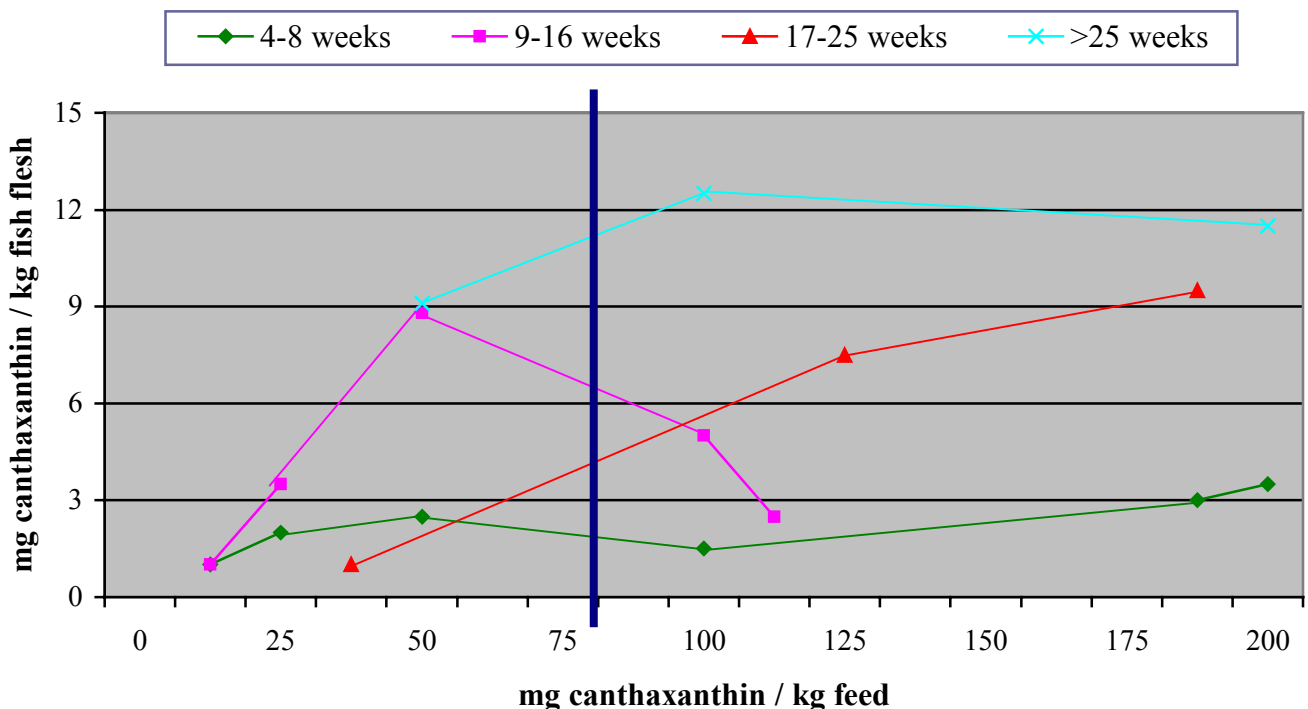
4.3.3. *Relationship between diet and flesh storage canthaxanthin concentration*

Studies on rainbow trout in freshwater (Choubert and Storebakken, 1989) and saltwater (Bjerkeng *et al.*, 1990) have shown that the canthaxanthin concentration in the flesh of immature trout increased when the dietary pigment concentration was increased from 0 to 200 mg carotenoids/kg of feed. However the response to dietary doses higher than 50 mg canthaxanthin/kg of feed was very low, mainly because carotenoid absorption is depressed when dietary concentration is increased (Choubert and Storebakken, 1989, Torrissen *et al.*, 1990).

The annex summarises for salmon and trout the current available data between diet (including feeding rate, duration) and tissue canthaxanthin concentrations indicating the final fish weight.

The figure 2 is an attempt to outline the relationship between diet and tissue canthaxanthin concentrations in the flesh of trout.

Figure 2. Canthaxanthin levels in the flesh of trout in relation with the dose and the duration of feeding, established on the basis of data presented in annex.



The vertical line in figure 2 indicates the current maximum permitted level of inclusion of canthaxanthin in feed (80 mg canthaxanthin/kg feed) as authorised in the Community legislation.

Although data do not come from the same experiments (see annex), lines have been drawn in figure 2 in order to allow an easier interpretation.

Different factors such as apparent absorption of dietary canthaxanthin in salmonids, interaction with vitamin A and vitamin E and sexual maturation affect canthaxanthin deposition in the flesh and explain the very wide variations observed:

5. USE OF CANTHAXANTHIN IN POULTRY

5.1. Consumer choice and food technology constraints

5.1.1. Egg yolk colour

In the EU, yolk colour is an important criterion for consumers choice of eggs. The colour is used as a tool to assess the quality of eggs. Yolk colour is indeed named at third position under egg quality traits (Hernandez and Blanch, 2000a, b). In addition to colour as such, homogeneity of yolk colour is important and associated with good quality. Egg yolk colour varies across Europe (see Table 1).

Northern countries, with the exception of Germany, prefer weakly coloured yolks, whereas countries of the South West of Europe prefer more intensively coloured yolks. The preferences for yolk colour of consumers vary also between regions within countries.

In addition, depending on the intended use of eggs, pigments are added to various extents to the hen diet.

Egg yolk colour directly reflects the concentration of pigments in the diets of laying hens. It is usually measured with special colour fans covering the different levels of coloration (from pale yellow to orange and red) and reflecting the different combinations of yellow and red carotenoids in the diets. The most used is the Roche Yolk Colour Fan (RYCF) displaying a scale from 1 (pale yellow) to 15 (reddish orange), designed hereafter by RYCF.

Due to the contribution of other carotenoids brought by various feed ingredients, but also to individual variations of the canthaxanthin physiological transfer process, it appears that a given RYCF score corresponds to a wide range of canthaxanthin concentrations.

Table 1. Approximate relationship between colour intensity and canthaxanthin content of egg yolk marketed in different European countries (measured with RYCF).

Country	RYCF score ¹	mg cantha/egg ²
Austria	12-14	0.16-0.35
Belgium	12-13	0.16-0.27
Denmark	9-10	0.09
Finland	9-10	0.09
France	11-12	0.13-0.16
Germany	11-14	0.13-0.35
Greece	11	0.13
Italy	12-13	0.16-0.27
The Netherlands	7-9	0.05-0.09
Portugal	12-14	0.16-0.35
Spain	11-14	0.13-0.35
Sweden	9-10	0.09
UK & Ireland	10-11	0.13

¹ Blanch (2000); ² based on Grashorn *et al.* (2000) data.

5.1.2. Poultry skin colour

In most European countries carotenoids are not used for pigmentation of skin in poultry for fattening, however, in some areas, consumers are interested in poultry carcasses with a coloured skin. Therefore feed components rich in natural carotenoids such as corn and marigold, as well as canthaxanthin, are used in diets. However no reference scale exists to compare skin coloration hues.

5.2. Practical conditions of egg and tissue colouring

5.2.1. Relationship between diet concentration and egg and tissue colouring

Pigmentation does not only depend on the total amount of pigment but also on the proportion of yellow and red carotenoids ingested. Low levels of red pigments added to diets with higher levels of yellow pigments result in a very intense yolk colour (De Groote, 1970), whereas supplementation of a weakly coloured yellow basis diet with a high level of canthaxanthin gives egg off-colours. Therefore in order to reach the wanted colour of the yolk, the addition of yellow pigments and canthaxanthin to a diet must take into account the original content of natural xanthophylls. Feed ingredients such as corn and alfalfa meal contain considerable amounts of yellow xanthophylls such as lutein and zeaxanthin. Red xanthophylls (capsanthin, capsorubin) are only found in paprika (*Capsicum annuum*, chilli) but show a pigmentation efficiency of about half to a third of that of canthaxanthin (Hyughebaert, 1993; Seemann, 1997; Grashorn *et al.*, 2000).

The effects of current levels of use of canthaxanthin in laying hens on coloration of fresh and boiled eggs have been investigated recently (Grashorn *et al.*, 2000)(see Table 2).

Table 2. Effect of dietary canthaxanthin levels on yolk colour of fresh eggs measured with RYCF (Grashorn *et al.*, 2000)

Red pigment: Canthaxanthin (in mg/kg feed)	Yellow pigment: carotenic acid-ester (in mg/kg feed)	RYCF	
		Fresh egg	Boiled egg
0.5	4.0	6	5
1.0	4.0	8	7
2.0	4.0	10	8
4.0	4.0	12	10
8.0	4.0	14	13

The visual colour score is reduced by at least 1 unit in yolks of eggs by boiling. Therefore, that loss is compensated through supply of an additional amount of yellow (2 to 4 mg/kg complete feedingstuff) and red (2 to 4 mg/kg complete feedingstuff) pigments to provide the desired coloration of yolks (Grashorn *et al.*, 2000). Braunlich (1974) achieved comparable results. The regression coefficients in the present case are 2.8 for fresh and boiled yolks and are consistent with data from literature (Schiedt, 1987; Hencken, 1992).

Recent trials performed by Sidibè (2001) give additional data on canthaxanthin deposition in the egg yolk after feeding canthaxanthin to laying hens at levels of 2-6 mg/kg feed. The colour intensity of the egg yolk reached a plateau after 10 days and the canthaxanthin levels in the egg yolk measured between day 19 and 25 reflect a stable relationship between canthaxanthin in feed and egg yolk.

Referring to the data compiled on Table 2, the SCAN considered that the average RYCF score in the countries demanding strongly coloured eggs was 13, but that the colour loss during egg processing implied to have an initial score of 14. The corresponding and highest canthaxanthin concentration found in the egg satisfying that score, *i.e.* 0.35 mg/egg or 5.9 mg/kg egg, was used in the calculation of the exposure.

5.2.2. Current practice

Practical layers' diets include distinct amounts of corn and alfalfa meal contributing to a content of native xanthophylls in the diet of 6 to 10 mg/kg. Diets low in native xanthophylls and therefore exhibiting low yellow pigmentation properties (Belyavin and Marangos, 1987), such as those rich in wheat or barley, are supplemented with yellow pigments (e.g. 4 mg/kg β -apo-8'-carotenoic-acid-ethylester) to avoid discolouration. In order to satisfy the range of colour scores required by the European egg producers,

natural or synthetic reddish carotenoids are added in both cases. Practically, the red component of the egg yolk colour is mostly brought by the addition of canthaxanthin at levels between 2 and 6 mg/kg complete feedingstuffs. Alternatively citranaxanthin can be used at a concentration 1.5 time higher than that of canthaxanthin to obtain a similar effect.

Generally, supplementation levels in layers feed may vary between 0 and 8 mg/kg feed for synthetic carotenoids for both yellow and red pigments, the sum of them amounting 10 to 15 mg/kg diet.

In the case of yellow broiler carcass, the concentration of pigment needed in the feed is higher than for laying hens. That is because the deposition rate is lower in the skin and subcutaneous fat than in egg yolk. The colour sought is yellow and in most of the case the pigments used are based on lutein (yellow hue) and zeaxanthin (orange hue) xanthophylls. The yellow skin of broilers is currently achieved by feeding chickens with a diet based on maize. Lutein and zeaxanthin are indeed present in maize, alfalfa, gluten meal, marigold (*Tagetes erecta*) and others. If a redder colour is sought then red pigments such as canthaxanthin or citranaxanthin are used. In the case of highly red pigmented broiler carcass, the relation between yellow hue and orange and red hue should be 3 mg of yellow (lutein) and 1,5 mg of orange (zeaxanthin) and also 1 of canthaxanthin. This means in normal practice that the canthaxanthin level in a normal diet could be between 2 and 6 mg/ kg of feed.

5.3. Deposition of canthaxanthin in the egg and tissues

5.3.1. Fate of canthaxanthin in poultry

Canthaxanthin is absorbed in the small intestine and transported *via* the blood to the liver. There, a part of the absorbed canthaxanthin undergoes metabolic change and is transformed into 4'-hydroxyechinenone and isozeaxanthin but also 4-oxoretinol, a vitamin A precursor, in both laying hens and broilers (Tyczkowski and Hamilton, 1986; Schiedt, 1998). The remaining unchanged canthaxanthin is transported by lipoproteins via blood to the target deposition sites. Less than 40% of the dietary canthaxanthin is deposited in egg yolk, whereas the deposition in the body tissues is lower than 10% (Schiedt, 1987; Hoppe and Krennrich, 1995). The distribution of the total radioactivity in the different tissues and organs following the repeated administration (8 mg/kg feed) of radioactive canthaxanthin to the hen is the following: ovaries (68-69%), liver (5.2-6.3%), muscle (3.2-7.5%), fat (1.0-1.2%), skin (1.1-1.1%) (Schiedt, 1987). Further studies carried out using radiolabelled canthaxanthin have allowed the isolation of metabolites from the liver of both laying hens and broiler chicks (Schiedt, 1990) as well as from egg yolk, spleen, kidney and perineal fat of layers (Schiedt, 1987). Unchanged canthaxanthin represented 40% of the total residues in the liver while the 4-oxoretinol was the major metabolite

(30%). The relatively low content of the reduction products 4'-hydroxy-echinone and isozeaxanthin is noteworthy, whereas these metabolites are present at a much higher concentration (30% for 4'-hydroxyechinenone) and in esterified form in the toe-web and in tegumentary tissues (Tyczkowski *et al.*, 1988). No radioactivity could be recovered in β,β -carotene, which was therefore not an intermediate in the conversion of canthaxanthin into vitamin A. Even if these biotransformations are limited, they may reduce the pigmenting properties of canthaxanthin in the hen (Hencken, 1992).

5.3.2. Relationship between diet and tissue canthaxanthin concentration

Concentrations of canthaxanthin in broiler chicken tissues are proportional to dietary levels and are lower than 10 % of intake (Table 3)(Fletcher *et al.*, 1986; Tyczkowski and Hamilton, 1986; Schiedt, 1987; Hoppe and Krennrich, 1995). Therefore, the regression coefficient for skin/subcutaneous fat *versus* dietary concentration is about 0.1 (Hencken, 1992). Braunlich (1974) reported levels of at least 50 mg pigments/kg diet to achieve a satisfying coloration. In practice 40 mg of yellow pigments may be used as a basic coloration to which canthaxanthin may be added in levels of 10 to 20 mg/kg. In conclusion, depending on the preferred coloration for poultry skin pigmentation, 3 to 6 times the supplementation level of canthaxanthin is used for yolk pigmentation corresponding to supplementation levels of 12 to 25 mg canthaxanthin/kg complete feedingstuff.

Table 3. Relationship between diet and tissue canthaxanthin concentration in the chicken for fattening.

Feed mg/kg	Skin + subcutaneous fat* (mg/kg)	Broiler carcass** mg/kg
1	0.1	0.01
5	0.5	0.05
10	1.0	0.09
15	1.5	0.14
20	2.0	0.18
25	2.5	0.23

* feed:skin ratio 1:0.1 (Hencken, 1992)

** 9 % of broiler carcass weight is skin (Orr *et al.*, 1984)

Very limited data are available concerning canthaxanthin residues in poultry liver. Schiedt (1987) has shown that the total radioactivity in the liver of hens that received tritiated canthaxanthin at a 8 mg/kg level in the diet for 14 and 28 days (1 animal per time point) corresponded to a concentration of 3.30 and 4.77 mg/kg respectively, and was by far superior to that measured in the muscle but at a lesser extent the skin and fat. Tyczkowski and Hamilton (1986) have shown a linear relationship ($y=0.1875x$, calculated from results presented as a graph) between the canthaxanthin concentration measured in the chicken liver (mg/kg) and that administered to the animals through

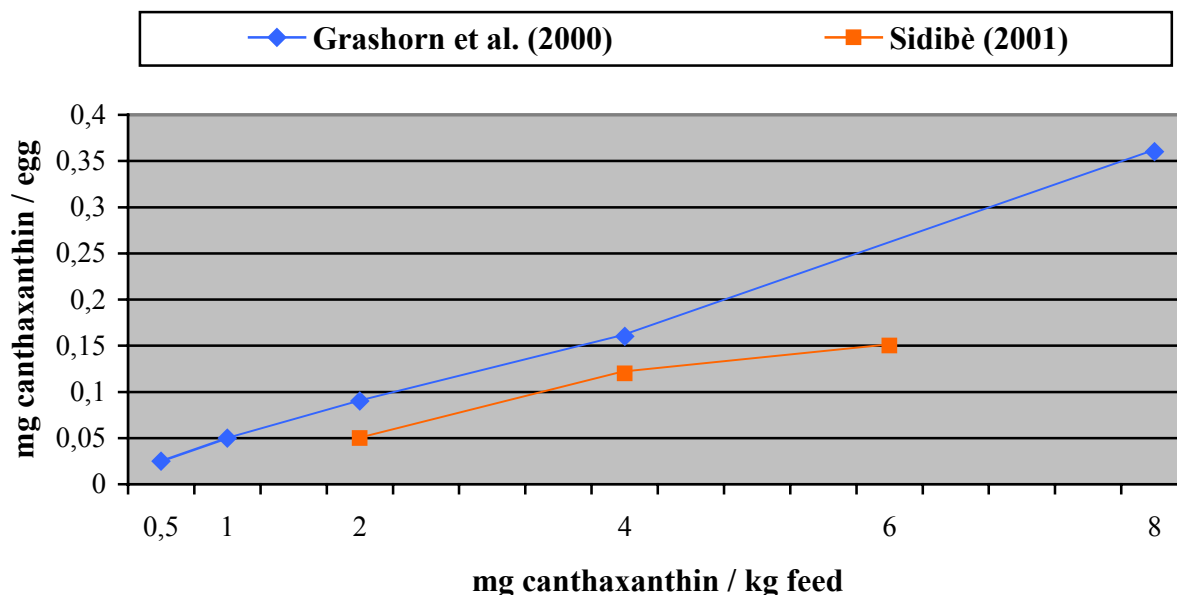
their diet (0, 5, 10, 20, 40 and 80 mg/kg, 10 animals per dose, 3 weeks duration). As the liver is not a typical storage organ for carotenoids, it can be considered that the residual concentration measured after the 3 weeks experimental exposure is representative of the canthaxanthin levels that would occur at the end of usual chicken production conditions (>42 days).

5.3.3. Relationship between diet and egg canthaxanthin concentration

The deposition of canthaxanthin in egg yolk is directly proportional to dietary level (Bornstein and Bartov, 1965; Braunlich, 1974; Tyczkowski and Hamilton, 1986; Grashorn *et al.*, 2000). However, when reaching high levels, this results in a lower deposition rate (Marusich *et al.*, 1974; Belyavin and Marangos, 1987; Puchal, 1988).

In poultry, there is a concentration effect as canthaxanthin accumulates predominantly in egg yolk. For a dietary canthaxanthin concentration of 1 mg canthaxanthin/kg complete feedingstuff, yolk canthaxanthin concentration reaches about 2.6 - 3.1 mg/kg egg yolk (Schiedt, 1987; Hencken, 1992; Grashorn *et al.*, 2000; Sidibè 2001) (see Figure 3).

Figure 3. Canthaxanthin in layer feed (mg/kg) *versus* canthaxanthin in the egg (mg/egg).



The deposition rate of canthaxanthin in yolks amounts to approximately 40 % for a canthaxanthin supplementation in the range of 0.5 to 8.0 mg canthaxanthin/kg diet (see Table 4). Braunlich (1974) reported a deposition rate of canthaxanthin of 2-3 mg/kg yolk for every 1 mg canthaxanthin/kg complete feedingstuff.

Table 4. Deposition rates of canthaxanthin supplements in the range of 0.5 to 8.0 mg/kg complete feedingstuff (Grashorn *et al.*, 2000)

canthaxanthin mg/kg feed	mg canthaxanthin uptake/egg	mg canthaxanthin/ egg	deposition rate (%)
0.5	0.060	0.023	38.3
1.0	0.114	0.051	44.7
2.0	0.253	0.087	34.4
4.0	0.395	0.163	41.3
8.0	0.920	0.352	38.3
Deposition rate = mg canthaxanthin per egg/mg dietary uptake of canthaxanthin per egg			

Different factors such as feed composition, carotenoids interaction and health status of the bird affect canthaxanthin deposition in the egg yolk and may explain the variability of the results observed:

6. ASSESSMENT OF CANTHAXANTHIN SAFETY

6.1. Estimation of canthaxanthin levels in products of animal origin in Europe

Published data on residue concentrations of canthaxanthin measured in the flesh of salmonids after different dietary canthaxanthin concentrations are reported in Annex. SCAN has chosen to use in its calculations the highest concentration resulting from the use of dietary levels complying with the current authorisation of canthaxanthin in feed as laid down in Council Directive 70/524/EEC, i.e. 13.7 mg canthaxanthin/kg trout flesh.

Concerning the eggs, the highest value of canthaxanthin residues determined experimentally (see 5.3.3.) in conditions mimicking the wider range of actual feeding practices was retained, i.e. 0.352 mg canthaxanthin/egg or, considering an average egg weight of 60g, 5.9 mg canthaxanthin/kg egg.

Based on the data available (see 5.3.2.) concerning canthaxanthin residues in chicken muscle and skin/fat determined experimentally in conditions mimicking the widest range of actual feeding practices, SCAN has retained the highest values, i.e. 0.23 mg canthaxanthin/kg muscle and 2.5 mg canthaxanthin/kg skin/fat. When the liver is considered, a residue concentration of 4.7 mg canthaxanthin/kg, corresponding to the same level of feed supplementation (25 mg/kg), has been chosen for the calculation of the exposure. The limited data available concerning kidneys (Schiedt, 1987) indicate that the total radioactivity in this organ is about one-third of that measured in the liver. Considering that the total radioactivity overestimates the unchanged canthaxanthin contribution and therefore represents a worse case than would be expected in practice, the SCAN has used a concentration representing the third of that chosen for the liver, i.e. 1.6 mg/kg kidney.

It must be noted that the SCF claimed (1997) that reliable estimates of canthaxanthin levels in food of animal origin were not available but that very limited data indicated they could be about 0.2 mg/egg and 0.1 mg/100 g fish. It appears that these anticipated values are well below those retained by the SCAN from the analysis of published experimental data (0.35 and 1.37 mg respectively) (see annex).

6.2. Assessment of the safety of canthaxanthin based on the maximum levels in fish and poultry retained by the SCAN

Considering the maximum canthaxanthin concentrations used above (6.1.) for fish, eggs and poultry, and using the daily human food consumption laid down in the Commission Directive 2001/79/EC fixing guidelines for the assessment of additives in animal nutrition, the SCAN calculated the daily exposure of a 60 kg mean body weight human for these food sources considered separately (Table 6).

Table 6. Theoretical intake of canthaxanthin by humans based on the SCAN approach and the maximum levels reported in food.

Product, tissue	Level in food (mg/kg) ⁽¹⁾	Assumptions		Canthaxanthin Daily Intake (mg/kg bw) for a 60 kg person
		Food consumption (g/person/day)	Canthaxanthin (mg/day)	
Egg	5.9	100	0.59	0.010
Birds ⁽²⁾	Meat (muscle)	0.23	300	0.069
	Skin + fat	2.5	90	0.225
	Liver	4.7	100	0.47
	Kidney	1.6	10	0.016
Fish muscle ⁽³⁾	13.7	300 ⁽⁴⁾	4.11	0.069

⁽¹⁾ Levels retained by SCAN based on published data.

⁽²⁾ Assuming all birds consumed are broilers

⁽³⁾ Assuming all fish consumed are salmonids

⁽⁴⁾ Value for consumption of fish muscle with attached skin in natural proportions.

Moreover, anticipating different situations where canthaxanthin would be used either for fish or poultry (laying hen and chicken), or for both applications, the SCAN calculated the corresponding theoretical consumer exposure and compared it with the ADI (Table 7).

It appears that in fish flesh canthaxanthin residues resulting from the administration of the pigment at a dietary concentration of 43 mg/kg, i.e. below the permitted maximum incorporation level (80 mg/kg feed), and for a 61-week period would be unacceptably high. A consumer eating a standard 300 g portion of fish containing such residues would receive an exposure to canthaxanthin in excess of the ADI. On the other hand, the residues in chicken and eggs after the administration of canthaxanthin at the highest level encountered in practice, i.e. 25 mg/kg and 8 mg/kg respectively, would give consumers intakes of canthaxanthin within the range of the ADI.

Table 7. Contribution of fish and poultry products to the ADI based on the SCAN approach.

	Canthaxanthin theoretical daily intake (mg/kg bw/day)	% ADI ⁽¹⁾
Allowed for fish only	0.069	228
Allowed for laying hen and chicken only (poultry products plus egg)	0.024	80
Allowed for fish, laying hen and chicken	0.079 ⁽²⁾	263

¹⁾ ADI = 0.03 mg/kg bw (SCF, 1997)

²⁾ the highest value corresponding to fish plus egg

6.3. Canthaxanthin levels in feedingstuffs and compliance with safety requirements

The SCAN has been asked to review the maximum levels of canthaxanthin in feedingstuffs for laying hens, other poultry, salmon and trout that would ensure the safety of the consumer, with particular regard to vulnerable groups within populations consuming foodstuffs containing canthaxanthin. It must be recalled that the assessment of the safety of feed additives, as laid down in the Commission Directive 2001/79/EC, is based on theoretical daily human food consumption values that overestimates the real figures in order to cover all vulnerable segments of the population and even extreme dietary habits.

Moreover the SCAN was asked to take into consideration the minimum level of the pigment in the product necessary to observe the technological effect.

6.3.1. Salmonids

The use of canthaxanthin in salmonids production leads to residues in the flesh that could expose some human consumers to amounts of canthaxanthin in excess of the ADI. This was the case even for feed concentrations (43 mg/kg) below the maximum permitted concentration (80 mg/kg). Based on the consumption figures used by the SCAN to calculate the human exposure, *i.e.* 300g fish flesh, the highest canthaxanthin concentration in the flesh that would assure a human exposure complying with the ADI (0.03 mg/kg bw) has been calculated. Considering that canthaxanthin would still be used for both salmonids and poultry production, the contribution of eggs to human exposure must be taken into account. Using the highest contribution of eggs (see 6.2.), *i.e.* 0.01 mg/kg bw, a maximum of 0.02 mg/kg bw can be allocated to canthaxanthin exposure related to fish consumption. This value corresponds to 4 mg canthaxanthin/kg fish flesh. Conversely, if canthaxanthin was permitted for salmonids only, the highest concentration in fish flesh that would comply with the ADI would be 6 mg/kg.

Referring to the current practice (see 5.1.) it appears that these concentrations confer to the flesh a colour hue, which is much lower than the actual market needs. If producers would still seek stronger colours, use of alternative carotenoids could be expected.

According to the data discussed previously (see 6.1.) the highest canthaxanthin concentration in fish feed that would lead to a maximum concentration of 4 mg/kg in the flesh would be 25 mg canthaxanthin/kg of feed for portion-size trout raised during 9-10 weeks. For salmon and large size trout (2 to 3 kg), that have been fed supplemented feed until slaughter (over 25 weeks), the variability of the results does not allow to set a dietary concentration of canthaxanthin that would ensure that residue levels in the flesh were below 4 mg/kg.

Considering the 6 mg/kg value (in the case of use of canthaxanthin for fish only), the figures would be (cf. annex) as follows: 25 mg canthaxanthin/kg of feed for salmon and trout (2 to 3 kg) until slaughter and 50 mg/kg of feed for portion-size trout.

6.3.2. Poultry

The human exposure to canthaxanthin resulting from the consumption of poultry products and eggs of animals fed the highest canthaxanthin dosages encountered in practice complies with the ADI. Therefore these values, *i.e.* 8 mg/kg for laying hen feed and 25 mg/kg for chicken feed (see 5.1.) can be used as the maximum permitted dietary concentration in place of the 80 mg/kg that is currently permitted.

7. CONCLUSIONS

Canthaxanthin is a carotenoid pigment used as feed additive for the sole purpose of colouring food:

- for salmonids, alone or together with astaxanthin, in order to obtain the reddish colour of the flesh of fresh and processed fish,
- for laying hens as a source of pigment to modify egg yolk colour in eggs for direct consumption or for use in food preparations.
- for broilers as a source of pigment to obtain a yellow hue of the skin.

In the European Union, canthaxanthin is currently authorised for use as a colouring agent up to a level of 80 mg per kg in complete feedingstuffs for salmonids and poultry. In the case of fish, when combined with astaxanthin, there is a maximum permitted level of 100 mg total canthaxanthin plus astaxanthin per kg complete feedingstuff.

7.1. The deposition of canthaxanthin in fish flesh, hen eggs and broiler skin/fat is related to the concentration of the pigment in the animal diet, but due to the decrease in the bioavailability of the pigment when its concentration in the diet increases, this relationship is not linear. Many nutritional, physiological and environmental factors are involved in the process of canthaxanthin deposition that explain the great variability of the results obtained in practice, especially for fish.

Based on published data, the requirements of the market in terms of organoleptic appraisal of raw but also processed foods can be achieved:

- for salmonids flesh: with a minimum concentration of 8 mg carotenoids/kg flesh, whatever the nature of carotenoids, *i.e.* canthaxanthin or astaxanthin, notwithstanding the fact that each of these pigments may contribute differently to the shade of the products,
- for the eggs: with a concentration of 6 mg canthaxanthin/kg egg,
- for broiler carcass: with a concentration of 2.5 mg canthaxanthin/kg skin/fat and concomitantly 0.25 mg canthaxanthin/kg muscle.

7.2. Canthaxanthin is the sole pigment of the carotenoid family registered for the use in both animal feeds and human foods. In the European Union, it is also the sole carotenoid pigment for which an Acceptable Daily Intake (ADI) has been established (0.03 mg canthaxanthin per kg body weight).

Maximum canthaxanthin contents in salmonids (13.7 mg/kg), eggs (5.9 mg/kg egg) and broiler skin/fat (2.5 mg/kg) resulting from the administration of supplemented feed complying with the actual EU authorised levels of inclusion in feed (up to 80 mg canthaxanthin/kg feed) have been retained by the SCAN based on the analysis of the published data. Considering these data the assessment of the safety of canthaxanthin for the human consumer indicates that the ADI is largely exceeded for fish (263%) but not for poultry (80%). It is noteworthy that the safety assessment that was laid down on the principles established in Directive 2001/79/EC overestimates the human food consumption in order to cover the potentially vulnerable segments of the population and extreme consumption habits.

7.3. On the basis of the available data, consumer safety would be assured by the setting of a maximum concentration of canthaxanthin:

- at 25 mg canthaxanthin/kg of feed for salmonids,
- at 25 mg canthaxanthin/kg of feed for broilers,
- at 8 mg canthaxanthin/kg of feed for laying hens.

These concentrations are well below the 80 mg/kg feed dosage that is currently permitted. For salmonids, this would result in a concentration of 4 mg/kg flesh in portion trout. This would not meet market needs.

Such a reduction in the maximum concentration of canthaxanthin permitted for use in fish feed would result in an increase in the use of authorised alternative colouring agents.

8. RECOMMENDATIONS

Considering that current levels of canthaxanthin in feed can cause residues in food products of animal origin at concentrations that could cause some consumers to have canthaxanthin intakes in excess of the ADI established by the SCF, SCAN recommends that the maximum permitted concentrations in feed be reviewed to ensure consumer safety.

If the Commission intends to lower the levels of canthaxanthin authorised in feed or to ban its use for some target animal species, it should anticipate the increasing use of substitutive or alternative substances (mainly astaxanthin in the case of salmonids and citranaxanthin in the case of poultry). Consequently, SCAN recommends that a risk assessment of the possible alternative colouring agents be made.

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10. ANNEX

Canthaxanthin deposition reported in the flesh of salmonids by different dietary canthaxantin concentration, feeding rate and duration of feeding.

Fish	Diet conc.	Feeding rate	Final fish weight	Duration	Average Flesh conc.	Reference
Species	mg/kg		g	week	mg/kg wet flesh	
Atlantic salmon	18	---	2200	74	6.3	Tidemann <i>et al.</i> , 1984
Atlantic salmon	30	to excess	406	56	0.5	Storebakken <i>et al.</i> , 1987
Atlantic salmon	43	to excess	1200	61	6.1 ± 2.0	Storebakken <i>et al.</i> , 1986
Atlantic salmon	46	to excess	1400	61	4.1 ± 0.6	Storebakken <i>et al.</i> , 1986
Atlantic salmon	60	to excess	406	56	3.6	Storebakken <i>et al.</i> , 1987
Atlantic salmon	90	to excess	406	56	3.6	Storebakken <i>et al.</i> , 1987
Atlantic salmon	100	---	1570	15	0.8	Storebakken <i>et al.</i> , 1984
Trout	12.5	3g/fish/day	235	6	1.1	Choubert and Storebakken, 1989
Trout	14	---	---	15	1.4	Tidemann <i>et al.</i> , 1984
Trout	25	3g/fish/day	235	6	1.8	Choubert and Storebakken, 1989
Trout	25	0.5-1.5%BW/day	210	8	2.1	Storebakken and Choubert, 1991
Trout	25	0.7-1.3%BW/day	900	16	3.6	Bjerkeng <i>et al.</i> , 1990
Trout	40	not given	175	24	1.2	Deufel, 1965
Trout	43	to excess	2900	61	13.7 ± 3.9	Storebakken <i>et al.</i> , 1986
Trout	46	to excess	3100	61	10.2 ± 1.6	Storebakken <i>et al.</i> , 1986
Trout	48	to satiation	150-200	9	2.6 ± 0.3	Torrissen, 1986
Trout	50	3g/fish/day	235	6	2.2	Choubert and Storebakken, 1989
Trout	50	0.5-1.5%BW/day	210	8	2.9	Storebakken and Choubert, 1991

Fish	Diet conc.	Feeding rate	Final fish weight	Duration	Average Flesh conc.	Reference
Trout	50	1%BW/day	495	9	10.0 ± 0.3	Pozo <i>et al.</i> , 1988
Trout	50	1.0-1.2%BW/day	1000	11	0.5 - 2.2	Röpke, 1988
Trout	50	0.7-1.3%BW/day	900	16	8	Bjerkeng <i>et al.</i> , 1990
Trout	50	not given	1950	26	4.8 ± 0.3 - 7.2 ± 0.5	Torrissen and Naevdal, 1984
Trout	100	3g/fish/day	235	6	2.5	Choubert and Storebakken, 1989
Trout	100	not given	not given	6	3.2 ± 0.2 - 2.8 ± 0.1	No and Storebakken, 1992
Trout	100	not given	not given	12	6.4 ± 0.3 - 7.3 ± 0.7	No and Storebakken, 1992
Trout	100	0.7-1.3%BW/day	900	16	8.7	Bjerkeng <i>et al.</i> , 1990
Trout	100	to excess	3612	140	3.9 ± 1.0 - 20.2 ± 1.8	Bjerkeng <i>et al.</i> , 1992
Trout	111	not given	540	14	2.4	Foss <i>et al.</i> , 1986
Trout	120	---	---	17	8.9 ± 1.6	Johnson <i>et al.</i> , 1980
Trout	192	to satiation	1190	5	3.0 ± 0.9	Choubert and Blanc, 1989
Trout	192	4.5mgCX/fish/day	2179	25	9.5 ± 0.8	Choubert and Blanc, 1989
Trout	200	to satiation	180	4	2.5 ± 0.2	Choubert, 1985b
Trout	200	to satiation	200	5	5.8 ± 1.6	Choubert, 1985a
Trout	200	3g/fish/day	235	6	2.8	Choubert and Storebakken, 1989
Trout	200	not given	160	52	11.4	Luquet <i>et al.</i> , 1983
Trout	207	to excess	123	8	1.4 ± 0.2	Torrissen, 1989
Trout	220	to satiation	180	5	9.2 ± 3.5	Choubert and Luquet, 1979
Trout	250	not given	264	8	2.5 ± 0.5	Abdul Malak <i>et al.</i> , 1975
Trout	800	---	---	---	18.3	Savolainen and Gyllenberg, 1970