EFFECTIVE USE OF CAROTENOIDS FOR SALMON FLESH PIGMENTATION

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Abstract

This presentation discuss pigmentation of Atlantic salmon in relation to factors influencing the pigment deposition and colour appearance of salmonid flesh, diet composition and dietary carotenoid level. The aim was to focus on methods to maximise the level of carotenoids in the flesh and to improve carotenoid retention. It is concluded that no beneficial effect is achieved by increasing the astaxanthin levels in the dry, extruded, diets above 60-70 mg/kg regardless of the energy density of the diet. Increased lipid level in the diet improve carotenoid retention, and increase muscle concentration. Astaxanthin is a more efficient pigment for salmon than canthaxanthin, but there are no apparent differences in the reflectance spectra.

Factors influencing the pigment deposition such as specific carotenoids, pigment sources, seasonal variations, stress and diseases are briefly mentioned.

L'UTILISATION EFFICACE DES CAROTÉNOÏDES POUR LA PIGMENTATION DE LA CHAIR DE SALMONIDÉS

Résumé

Cet article traite de la relation entre la pigmentation du saumon Atlantique et les divers facteurs qui influencent l'accumulation de pigments et la coloration de la chair des salmonidés, les effets de la composition de la diète et de la concentration en caroténoïdes de la ration. L'objectif était de s'attarder sur des méthodes qui permettent de maximiser la concentration de caroténoïdes dans la chair et d'améliorer la rétention des caroténoïdes. Les conclusions indiquent qu'il n'y a aucun avantage à augmenter la concentration d'astaxanthine au-delà de 60 à 70 mg/kg dans une ration sèche agglomérée, indépendamment de la densité énergétique de cette ration. Une augmentation de la teneur en lipides de la ration favorise la rétention de caroténoïdes et en accroît la concentration musculaire. L'astaxanthine est un pigment plus efficace pour les saumons que la canthaxanthine, mais aucune différence apparente n'est observée dans le spectre de réflectivité.

Les facteurs qui influencent l'accumulation de pigments, tels que les caroténoïdes spécifiques, les sources de pigments, les variations saisonnières, le stress et les maladies sont discutés brièvement.

Introduction

The production of Atlantic salmon world-wide exceeded 460,000 tonnes in 1995. This salmon was fed 25-30 tonnes of pure astaxanthin and canthaxanthin in order to achieve a satisfactory flesh pigmentation of approximately 8 mg/kg salmon flesh. In Norway, this astaxanthin supplementation represents between 20 and 25 % of the total feed costs or approximately 8-10 % of the total production costs.

Second after freshness, a sufficient pigmentation is regarded as the most important quality criteria for farmed Atlantic salmon (*Salmo salar*) (Koteng, 1992). The requested flesh pigmentation has increased the last years. An astaxanthin level in the range of 1.5-3 mg/kg was accepted during the 1970ies, but the required level has steadily increased, and today a astaxanthin level above 8 mg/kg is necessary in order to achieve market acceptance. This represents about 16 on the Roche Color Card Score. The desire of a *Oncorhynchus* like pigmentation explains this contest in achieving the highest possible carotenoid deposition in the Atlantic salmon flesh. The principal solution has been to increase the dietary supplementation of astaxanthin, and feeding astaxanthin fortified diets during the whole production cycle. The amount of astaxanthin fed to the Atlantic salmon exceeds in many cases the amount the fish is able to utilise efficiently.

In my opinion it would be more beneficial to: A: Optimise the carotenoid retention in order to reduce the total pigmentation costs; B: to develop methods for controlling the carotenoid metabolism in order to achieve a higher flesh level of astaxanthin and C: to develop a pigmentation regime that minimise the variation in flesh pigmentation both between individual fishes and within the individual fillet.

Pigment sources

Carotenoids

Astaxanthin (Figure 1) is the primary salmonid carotenoid, and more than 95% of the carotenoids found in the flesh of wild salmon is astaxanthin. The astaxanthin in the flesh originate from ingested zooplankton or fish that have zooplankton in the digestive tract. It is suggested that incorporation of hydroxyl groups into the carotene skeleton increase the absorption in gold fish (Hata and Hata, 1972). For salmonids it is found a preferred deposition of astaxanthin followed by adonirubin and canthaxanthin. However, lutein, zeaxanthin and -carotene are poorly absorbed (Schiedt et al., 1985). Choubert et al (1995) reported apparent digestibility coefficients (ADC) for astaxanthin in the range of 75-57% in rainbow trout (*Oncorhynchus mykiss*), while less than 10% of the canthaxanthin was digested. The values for canthaxanthin are improbable low. Carotenoids are easy oxidised, the continuos collection of and freeze drying of the faeces, as done by Choubert et al (1995), may give an overestimation of the digestibility. This can explain the relative high ADC value they reported for the astaxanthin. Storebakken et al reported apparent digestibility values for astaxanthin in Atlantic salmon in the range of 45-74%, Astaxanthin dipalmitate of 39-42 and canthaxanthin 57-67%. Based on these results there are only a slight difference in digestibility coefficients between astaxanthin and canthaxanthin.

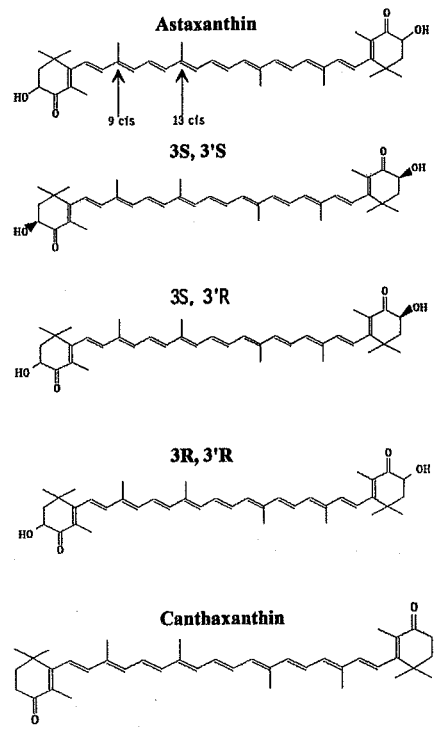

Figure 1. Astaxanthin (geometrical and optical isomers) and Canthaxanthin.

Canthaxanthin (Figure 1) is deposited in the flesh of salmon at an efficiency ratio of 0.6 compared to astaxanthin (Torrissen, 1989). This is substantially lower than the ADC differences suggests.

The absorption maximum for astaxanthin and canthaxanthin in n-hexane are similar, 470 and 476 respectively, but canthaxanthin is reported to give a red pigmentation with a more brown tone compared to a pink colour tone for astaxanthin pigmented flesh. However, reflectance measurements on Atlantic salmon fillets pigmented by astaxanthin, canthaxanthin or a mixture of astaxanthin and canthaxanthin showed no significant differences (Figure 2), but the canthaxanthin pigmented fish had a slightly higher reflectance between 550 and 600 nm.

Table 1.
Distribution of optical isomers in different astaxanthin sources.

Pigment source	3R,3'R	3R,3'S	3S,3'S
Syhtetic astaxanthin	**	****	**
Yeast (*Phaffia rhodozyma*)	******	*	*
Arctic shrimp (*Pandalus borealis*)	*	*	******
Krill (*Euphanasia superba*)	******	*	*
Algae (*Haematococcus pluvialis*)	*	*	******
Homarus spp.	*	*	******

Atlantic salmon seems not to be able to perform epimerization of the optical isomers of astaxanthin, and there are no evident differences in the absorption and deposition of the individual optically active isomers, (3R,3'R), (3S,3'S) and (3R, 3S) (Foss et al., 1984; Storebakken et al., 1984). Determination of the relative amount of optical isomers of astaxanthin are used as a tool for identification of escaped Atlantic salmon and eggs of escaped Atlantic salmon. Distribution of the optical isomers in different pigment sources are shown in Table 1.

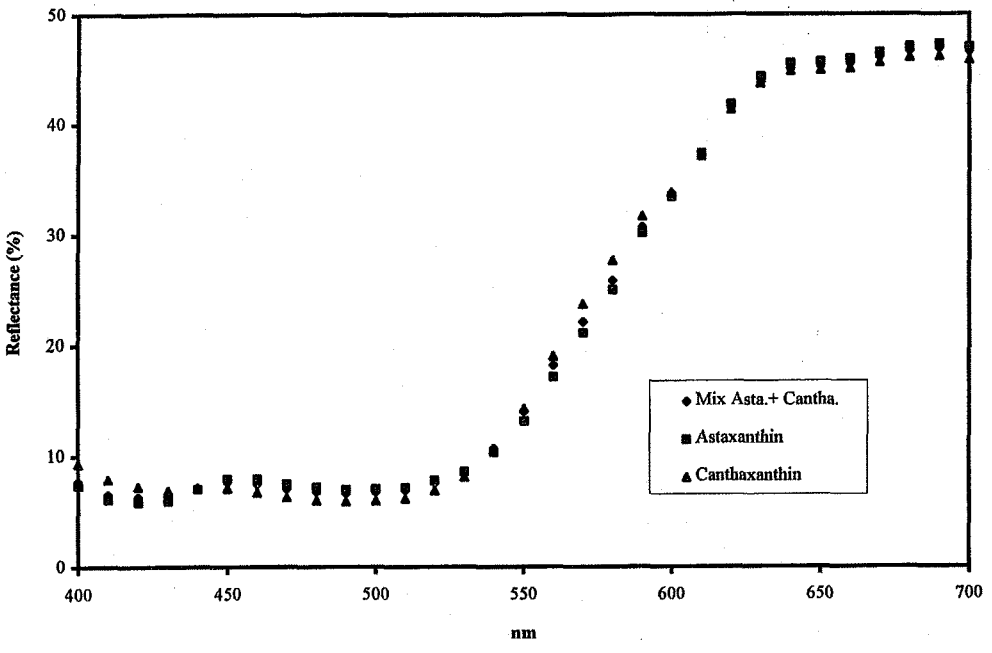

Figure 2. Reflectance of Atlantic salmon fillets pigmented by a mixture of astaxanthin and canthaxanthin, pure astaxanthin and pure canthaxanthin.

Cis carotenoids (9-cis and 13-cis) (Figure 1) exhibit light absorption of lower intensity than their all-trans isomer, E1%,1cm= 1750, 1310 and 2100 in n-hexane respectively (Hoffmann-La Roche, 1992). As a general rule, the light absorption maximum for cis-isomers are shifted to shorter wavelength (2-5 nm). In addition, a subsidiary peak in the near ultra violet region appears with a difference of 140 nm. I have no information on absorption and deposition of cis isomers of astaxanthin or canthaxanthin in fish, but pigment sources used in salmonid diets contain considerable amounts of cis isomers, sometime up to 20%.

Esters of astaxanthin is generally less utilised than the free form (Torrissen and Brækkan, 1979). However, the astaxanthin esters from zooplankton and shrimp waste seems to have a higher retention than the purified esters, this may be due to esterase's in the pigment source.

Astaxanthin and Canthaxanthin sources

Astaxanthin and canthaxanthin are available as synthetic products stabilised in gelatine beadlets containing 8 or 10% of the pure carotenoid. More than 90% of the farmed salmonids are pigmented by the synthetically astaxanthin or canthaxanthin. Other pigment sources are red fish oil, cray fish extracts, the yeast *Phaffia rhodozyma* and the algae *Haematococcus pluvialis*, but only Phaffia rhodozyma seems to have a potential as a significant pigment source, besides the synthetical products.

The red fish oil contain astaxanthin form zooplankton in the digestive tract of fish processed for fish meal and oil. The use of this is hampered by a low astaxanthin content and irregular supplies. Also the use of cray fish extracts is limited by the quantities available.

Strains of the yeast *Phaffia rhodozyma* may contain more than 10 g astaxanthin/kg dry yeast. The astaxanthin from the yeast seems available for salmonids at comparable rate to the synthetic products (Torrissen, unpublished). The use in commercial feeds is limited by availability in the market and price.

The astaxanthin in the *Haematococcus pluvialis* is mainly presented as esters. These astaxanthin esters is absorbed poorly by Atlantic salmon (Torrissen, unpublished), and a commercial use of this algae requires a hydrolysis of the ester bond prior to feed addition.

Colour impression

The pigmentation of salmon flesh can be evaluated by chemical analyses of the carotenoid content or by the estimating the appearance of the colour by visual judgement or reflectance colourimetry. Humans are in general good in comparing colour, but poor in remembering colour. Differences in colour between fillets and within the same fillet are a larger problem for the industry than the actual pigment level.

The methods used for visual determination of salmonid flesh colour have included trained sensory panels and the use of a reference colour card. A colour card for measuring the colour of raw salmon flesh was developed Hoffmann La Roche (Basle, Switzerland), the Roche Colour Card. This Roche Colour Card is widely used by the industry to evaluate the colour intensity of salmon.

Reflectance measurements of colour have been used in many areas such as colour control of printed matter, paints, textiles, glass and ceramics and also in the food industry. This include application for quality control of flesh and skin pigmentation of salmonids. The measurements can either be done directly on the fillet or on homogenised flesh samples, using the colour parameters lightness (L*), the red/green chromaticity (a*) and the yellow/blue chromaticity (b*) according to the recommendations of the International Commission on Illumination, CIE (1976). The quantitative Hue (H°_{ab}) is calculated from the measured chromaticity values (Hunt, 1977), and hue is expressed as the relation between the yellowness and redness of the fillet ($H^\circ_{ab}=\tan^{-1}(b^*/a^*)$). The relation between the Hue, L*, a* and b* and the astaxanthin concentration are not linear and reach a plateau at high astaxanthin concentrations (Figure 3). A non-linear regression line were calculated for the relation between the a* value and the astaxanthin concentration in the flesh;

$a^* = 3.51 + 3.64 \times Ln\ (Ax)$ where Ax = astaxanthin level in the flesh (Figure 2)
(R^2=0.974) (Christiansen et al.,1995.)

A high correlation is found between the Roche Colour Card score and the mean astaxanthin concentration in the flesh of a salmon population (R^2=0.99). In general, the Roche Colour Card give a better prediction of the astaxanthin

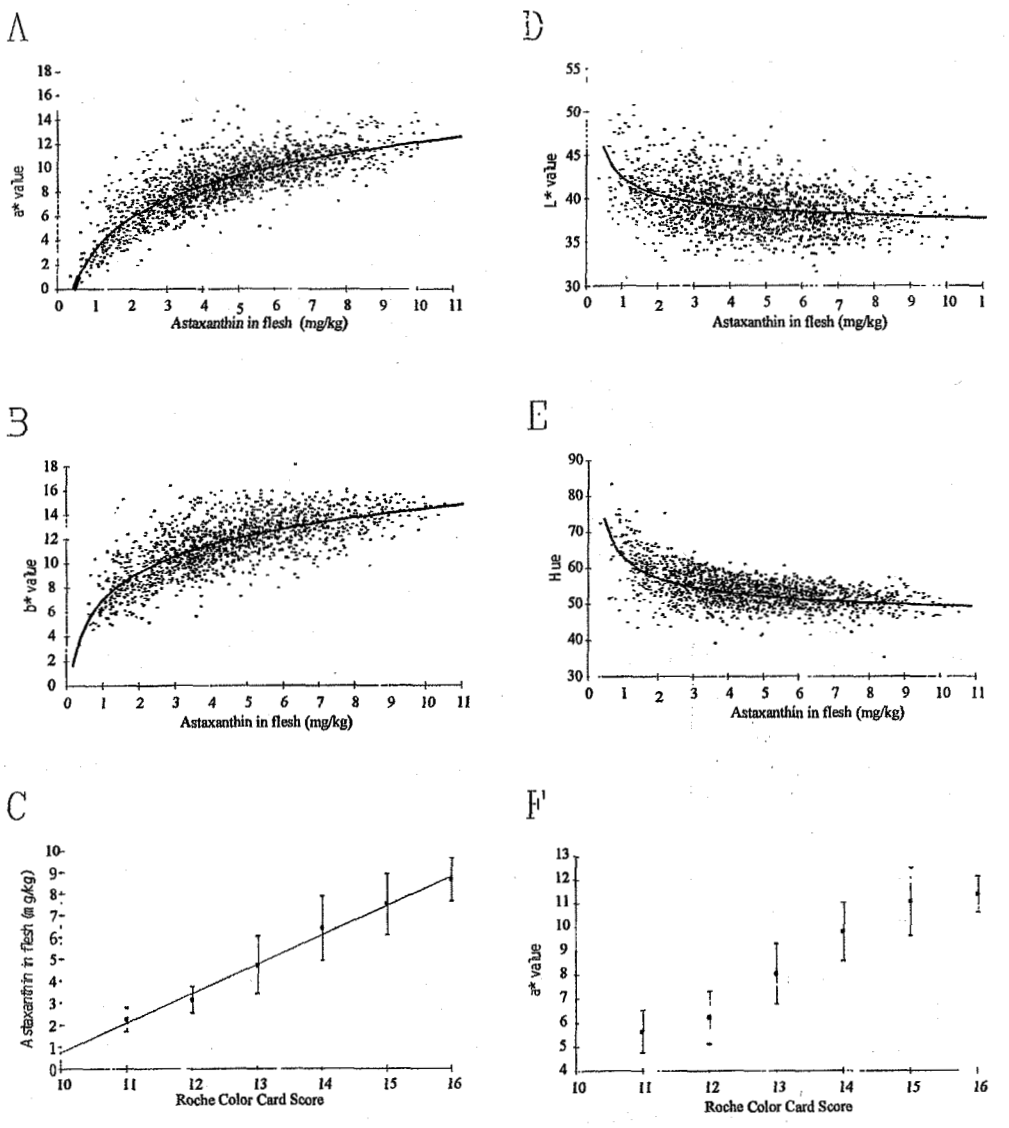

Figure 3. Relations between A: the red/green chromaticity (a*) and astaxantin concentration in the flesh; B: the tellow/blue chromaticity (b*) and astaxanthin; C: Astaxanthin in the flesh and the Roche Color Card Score; D: Lightness (L*) and astaxanthin in felsh; E: Hue (H^o_{ab})($H^o_{ab}=\tan^{-1}(b^*/a^*)$) and astaxanthin in flesh; F: a* values ant the Roche Color Card Score. (Christiansen et al., 1995)

concentration at higher astaxanthin levels than reflectance colourimetry when used under standard light condition and by trained staff. However, none of the methods gave a good prediction of the carotenoid concentration in single fillets, and all require a standardised sampling and measuring technique as they are very sensitive for factors affecting the surface of the fillet.

Metabolism of carotenoids.

The amount of carotenoids retained in the flesh are an equilibrium between the amount ingested, the digestibility of the carotenoids and the metabolism (Figure 4): ***Ingested carotenoids= Undigested + catabolised + retained.*** In general, there has been an underestimation of the effect of the metabolism of carotenoids on the pigment retained in the flesh. The mobilisation of flesh carotenoids during maturation, the lack of ability to deposit carotenoid in the flesh of juveniles below 3-400 g, and the quantitatively low reductive metabolism have been the factors of interest. However, we have evidence for an active oxidative metabolism of carotenoids in the liver, and excretion of the metabolic

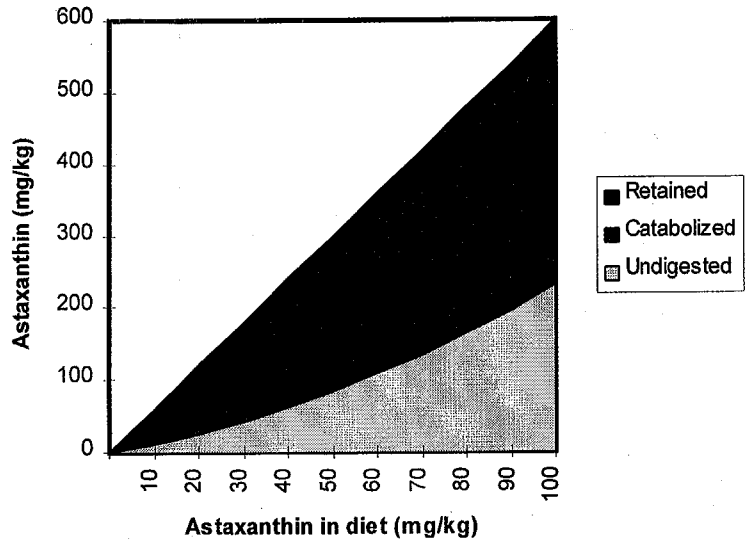

Figure 4. Estimation of astaxanthin fate in Atlantic salmon. The estimations are based on a 5 kg salmon, a Feed Conversion Rate of 1,2 and the values reported for retention by Torrissen et al., 1995 and apparent digestibility factors by Torrissen et al., 1990 and Choubert et al., 1995.

products through the bile. According to our calculation this catabolism oxidise near 50% of the ingested carotenoids (Figure 4).

Little information is available on factors controlling this metabolism, but environmental stress, diseases, fish size, and season have definite effects.

Our hypothesis is that the astaxanthin catabolism is the major factor controlling the maximum astaxanthin level in the flesh of salmonids. We also believe that differences in catabolic activity may explain the different ability among marine fish to deposit astaxanthin in the flesh. This is supported by the fact that white fish also absorb carotenoids efficient. In order to increase the level of astaxanthin in the flesh above the apparent plateau of approximately 8 mg/kg a method to reduce the catabolic activity of astaxanthin seems to be required.

Diet composition

The dietary factors of principal importance for astaxanthin absorption and deposition are the lipid level and astaxanthin concentration in the feed. In addition it has been speculated on negative effects of pro-oxidants and high dietary levels of other carotenoids and Vitamin - A, and a positive effects of antioxidants and phospholipids. It is, in a study by Rørvik et al (1992), showed that high iron levels in the diet gave a lower relative redness measured by a Minolta Chromometer. A corresponding inverse relationship were also found for the astaxanthin concentration in the flesh and the iron concentration in the flesh. This study does not however give any indications of the mechanisms for this relationship. The losses may occur during process and storage of the feed, by increased oxidation or catabolism in the live salmon or during sampling, storage and the astaxanthin determinations.

Dietary lipid level.

Increased dietary lipid level is reported to have a positive effect on the apparent digestibility coefficient of canthaxanthin in rainbow trout: ADC=0.869*%F – 2.038 (R^2=0.83)(Torrissen et al., 1990). However, increased dietary lipid levels increase the energy density of the diet, and will thus give a lower food conversion ratio (FCR). A higher canthaxanthin or astaxanthin concentration in the diet is thus required in order to compensate for the lower amount of

feed required per unit of growth. The increased ADC will more than compensate for the lower FCR, giving a theoretically increased consentration of carotenoids in the flesh.

Christiansen and Torrissen (unpublished) fed juvenile Atlantic salmon of initial weight 180 g extruded diets containing 3 different lipid levels (5, 18 and 25%) and 56 mg astaxanthin/kg for 151 days. The final concentration of astaxanthin in the flesh increased linearly by increasing dietary lipid level (Figure 5), this highly significant even after correction for differences in growth.

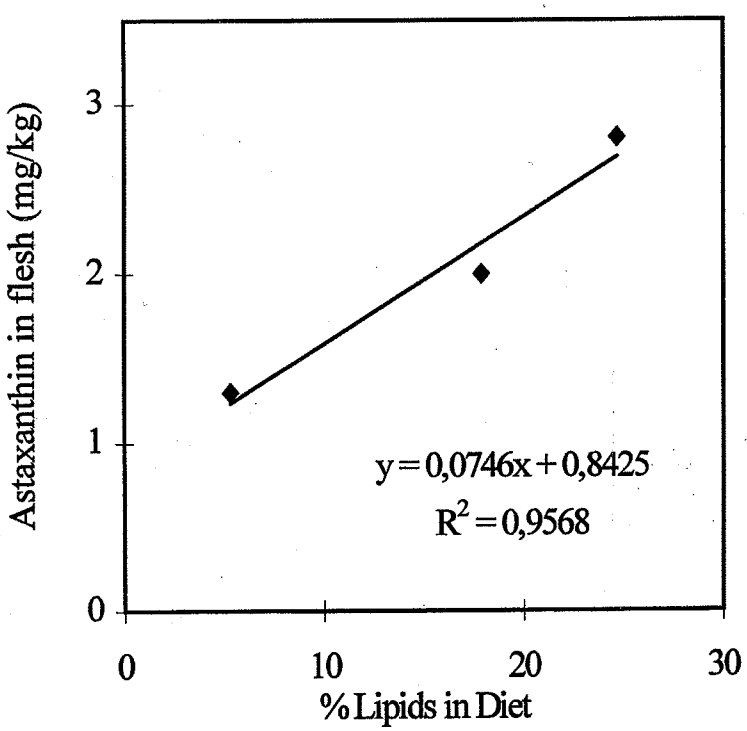

Figure 5. Astaxanthin in Atlantic salmon flesh after feeding 3 dietary lipid levels for 151 days. The astaxanthin level in the diets were 56 mg/kg (Christiansen and Torrissen, unpublished)

Astaxanthin seems to be associated with proteins in the flesh (Hemni et al, 1989). The protein level in salmon muscle are relative constant over a wide range of lipid concentrations. Flesh astaxanthin will thus not be diluted by increased amount of fat in the fillet. On the contrary, astaxanthin seems to increase significantly by higher fat levels in the flesh (Torrissen et al., 1995). However, large about of visual fat in the fillet will cover the pigmented flesh, and the fillet may look paler.

Dietary carotenoid concentration

The carotenoids have to be supplied through the diet, and in commercial diets the carotenoid level normally ranges from 40 to 100 mg/kg. Several reports have shown a relative sharp decline in the carotenoid deposition rate by increasing dietary carotenoid concentration (reviewed by Torrissen et al., 1989). This can partly be explained by a decreased digestibility of astaxanthin or canthaxanthin as the dietary level increase.

Torrissen et al (1995) fed Atlantic salmon 9 experimental diets containing from 0 to 200 mg astaxanthin per kg for 6 time periods ranging from 3 to 21 months in sea cages at Matre Aquaculture Research Station, Norway. The sampled fish had an initial mean weight of 115 g and reached a weight of 3.2 kg at the termination of the experiment.

Every third month 10 fish from each dose and time group were sampled and the astaxanthin concentration in the flesh determined. The amount of astaxanthin in the flesh ranged from 0.7 to 8.9 mg/kg at the termination of the experiment.

Figure 6 shows the calculated relation between dietary astaxanthin level and the pigment level in the flesh for the fish fed astaxanthin during the whole production cycle. The regression equation $Y=8.48*[1-e^{(-0.0603*X)}]$ shows a plateau in muscle astaxanthin concentration for Atlantic salmon of 3-3.5 kg fed astaxanthin fortified diets containing 23% fat and above 60 mg astaxanthin per kg feed for the whole production cycle of 8.5 mg/kg muscle. This value is close to the maximum astaxanthin level obtained in commercial Norwegian salmon production. It can on the basis of this experiment be concluded that increasing the dietary astaxanthin concentration above 60 mg/kg have no significant effect on muscle pigmentation of Atlantic salmon. It can also be concluded that, in order to achieve a maximum flesh pigmentation, a constant astaxanthin level in the feed of 60 mg/kg is more economically favourable than strategies exceeding the maximum bio-available astaxanthin concentration in the diet, 60 mg/kg.

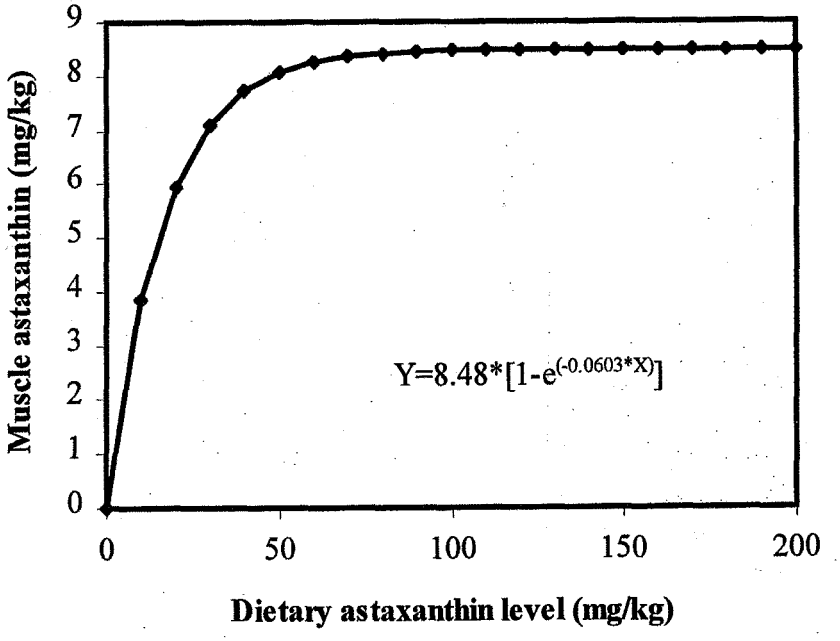

Figure 6. Astaxanthin in flesh of Atlantic salmon in relation to dietary astaxanthin concentration. The regression equation is based on data from fish fed astaxanthin during the whole production cycle.

As long as the astaxanthin supplied by the feed both has to pigment the growth as well as the already existing fillet mass, the relation between astaxanthin concentration in the flesh (Y) and weight (X) has to follow a general logistic model $Y=b_1*(1-\exp^{(b2*X)})$, where b_1 is the maximum obtainable astaxanthin concentration in the flesh. This factor is mainly influenced by the bio-availability of the carotenoids fed. The negative value of b_2 will approach 0 as the start weight for pigmenting the fish increases, this reducing the expected astaxanthin level in the fish flesh. This model illustrated the importance in starting the pigmentation as soon as the smolts are released to the sea pens in order to obtain a maximal pigment level in the fish.

The same study also demonstrated a drop in flesh astaxanthin accumulation during the spring months. This coincides with the time period when Norwegian farmers face problems in achieving a satisfactory pigmentation. A hypothesis is that this drop are under hormonal control. Illumination of the cages during the winter have eliminated the drop, and resulted in higher muscle pigmentation.

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