Aquat. Living Resour., 1993, **6**, 163-168

Muscle pigmentation changes during and after spawning in male and female rainbow trout, *Oncorhynchus mykiss*, fed dietary carotenoids

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Received September 14, 1992; accepted March 29, 1993.

Choubert G., J.-M. Blanc. *Aquat. Living Resour.*, 1993, **6**, 163-168.

Abstract

The dynamics of astaxanthin and canthaxanthin were studied through an 11-month feeding experiment by following carotenoid deposition in male and female rainbow trout muscle, during and after spawning. Carotenoids were analysed by chromatography (HPLC), muscle colour was measured by a colourimeter and visual rank evaluation. In both sexes, a decrease in the muscle carotenoid content was observed at spawning time. The recovery of the muscle pigmentation differed between females and males, but seemed not to be influenced by the type of carotenoid additives in feed. The carotenoid content of the female muscle increased rapidly after spawing, while in the male this increase was slower. Eighteen weeks after spawing, carotenoid concentrations in the muscle of females corresponded to those before spawning, while in males they reached only one third of that amount. Significant correlations were found between chemical analysis, colourimetric measurements and visual rank evaluation.

Keywords: carotenoid, pigmentation, colour, spawning season, trout.

Modifications de la pigmentation musculaire, lors de la reproduction, chez le mâle et la femelle de truite arc-en-ciel, Oncorhynchus mykiss, *recevant un aliment supplémenté caroténoïdes.*

Résumé

Les dynamiques de fixation de l'astaxanthine et de la canthaxanthine ont été suivies, pendant et après la période de reproduction, dans le muscle de truites mâles et femelles. La durée de l'expérience a été de 11 mois. Les caroténoïdes ont été analysés par chromatographie (HPLC), la couleur du muscle a été mesurée à l'aide d'un colorimètre et d'un test de rang. Pour les deux sexes, on a observé une diminution de la concentration en caroténoïdes du muscle au moment de la ponte. La reprise de pigmentation a été différente chez les mâles et chez les femelles, mais ne semble pas avoir été influencée par les caroténoïdes alimentaires. Alors que, chez les femelles, l'augmentation des caroténoïdes musculaires s'est faite juste après la ponte, chez les mâles, cette reprise a été plus lente. Dix-huit semaines après la ponte, les concentrations en caroténoïdes des femelles correspondaient à celles observées avant la ponte; chez les mâles, ces concentrations n'atteignaient que le tiers. Des corrélations significatives ont été trouvées entre l'analyse chimique, la mesure de la couleur et le test de rang.

Mots-clés : caroténoïdes, pigmentation, couleur, reproduction, truite.

INTRODUCTION

Because salmonids – salmon and trout – cannot synthesize carotenoids *de novo*, they must obtain them from their diet. In the wild, salmonids obtain most of their carotenoids by feeding on small crustaceans and other invertebrates. In intensively farmed fish the aquatic environment does not contribute sufficient carotenoids. These must be supplied as compounds produced by synthesis and added to the diet to meet the animal's requirement. Today, astaxanthin (3,3'-dihydroxy-4,4'-diketo-β,β-carotene) and canthaxanthin (4,4'-diketo-β,β-carotene) are used in salmonid feed. However, astaxanthin, the natural carotenoid of wild salmonid, is better deposited than canthaxanthin in the muscle of salmonids (Foss *et al.*, 1984; Torrissen, 1986; Choubert and Storebakken, 1989; Torrissen, 1989; Bjerkeng *et al.*, 1990; No and Storebakken, 1992).

Sexual maturation of salmonids involves significant changes in their metabolism, particularly in carotenoids (reviewed by Choubert, 1986). Wild salmonids stop feeding during anadromous spawning migration. Compared to immature fish, spawning fish have lower carotenoid concentrations in the muscle which may be explained by internal redistribution of carotenoids: astaxanthin would be mobilized from the muscle and accumulated in females in the ripening eggs, and in males in the skin (Steven, 1949; Crozier, 1970; Shnarevitch and Sakhnenko, 1971; Kitahara, 1983; Ando, 1986). Farmed salmonids are regularly fed during spawning season, although at a slightly lower rate. Muscular pigmentation of female trout has been shown to increase throughout the spawning season with dietary canthaxanthin at a level as high as 200 mg/kg diet (Choubert and Blanc, 1989). For males, no such data are available in the literature.

The objective of this study was to investigate the changes in pigment concentrations in the muscle of rainbow trout of both sexes, continuously fed astaxanthin – or canthaxanthin – supplemented diets, associated with spawning and post-spawning recovery. Colour of trout muscle was also investigated.

MATERIAL AND METHODS

Experimental conditions

The experiment was conducted in the INRA experimental fish farm of Lées-Athas (South-west of France). Four groups of 200 rainbow trout, *Oncorhynchus mykiss* (Walbaum), aged 2+ with an initial weight of 600 g were grown with 4 different diets in 4 separates 35 m^2 gravel bottom ponds. The water was taken from a mountain spring, having a constant temperature of 8°C (± 1°C). Its pH was 7-8 and it was saturated with oxygen (10 to 12 mg O_2/l). The experimental period lasted 11 months, beginning 30 weeks before spawning and ending 18 weeks after spawning.

Feed and feeding

Fish were fed a commercially available dry pelleted diet without carotenoid (proximate analysis: 49.2% crude protein, 8.7% crude fat and 1.15 kJ/g energy) as control (CTL). Astaxanthin or canthaxanthin was added as gelatin stabilized beadlets (Hoffmann-La Roche and Co, Basel, Switzerland) so as to obtain three experimental diets: A 50 and A 100 = control diet supplemented with 50 and 100 mg astaxanthin/kg diet respectively and C 100 = control diet supplemented with 100 mg canthaxanthin/kg diet. The analyzed carotenoid contents of the diets were, on a dry weight basis, CTL = 0 mg/kg, A 50 = 49.1 mg astaxanthin/kg diet, A 100 = 107.1 mg astaxanthin/kg diet and C 100 = 97.4 mg canthaxanthin/kg diet. All diets were stored at +4°C during the experiment. The fish were fed to apparent satiation twice a day during the 11-month experimental period.

Analytical methods

Five sampling operations were performed: ten weeks before spawing, at spawning, and 4, 8 and 18 weeks after spawning. At each sampling, muscles from 5 trout of each sex per group were removed. One fillet from each fish was used for visual rank evaluation and physical colourimetric measurement. The other fillet was kept at −80°C until carotenoid analysis.

Carotenoid analysis was carried on pooled samples of each sex within each experimental group. Muscles were blended for 30 s at maximum speed (approx. 1 500 r. per min). After acetone extraction, astaxanthin and canthaxanthin analysis were performed with an isocratic reverse phase high pressure liquid chromatography (RPHPLC) method (Guillou *et al.*, 1993). Carotenoid amounts were expressed on a dry basis using extinction coefficients (E 1% 1 cm) of 2 100 for astaxanthin and 2 200 for canthaxanthin, both at their absorption maximum in hexane (De Ritter and Purcell, 1981). One pigment extract was made from each sample, with two replicate carotenoid determinations from each pigment extract. Homogenates of the muscle samples from the fish selected for processing and colour analysis were analyzed for dry matter at 105°C overnight.

Colour of individual chopped muscle was measured according to Wyszecki and Stiles (1967) through the mouth of the cap on a colour analyser (Gardner LX 20, aperture 8 mm) calibrated with a white plate reference standard before each sample. Reflected light values were obtained by averaging four readings, the sample being rotated 90 degrees between each measurement (Schmidt and Cuthbert, 1969). From the redness (*a*) and yellowness (*b*) intensity values, the

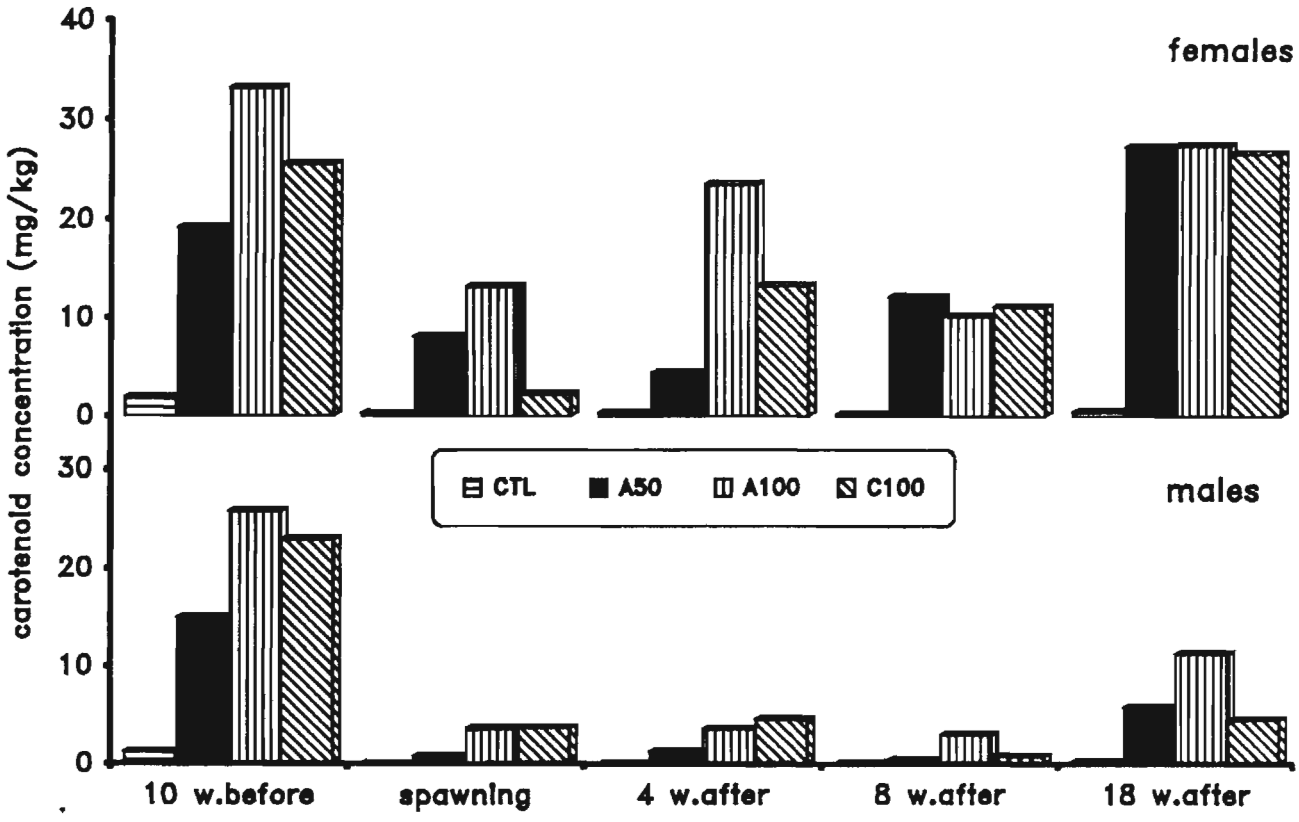

Figure 1. – Changes in muscle carotenoid concentrations associated with spawning and post-spawning recovery in male and female rainbow trout.

hue was calculated as 100 *a/b*, according to Wyzecki and Stiles (1967).

Visual evaluation was performed immediately after each sampling operation by ranking, under natural (outdoor) light, the 40 fillets from the lowest (rank 1) to the highest (rank 40) intensity of colour. Therefore, the evaluation for one fish was relative to each other fish within sampling dates and did not allow comparisons among successive samplings.

Data on pigment concentration and colourimetry were processed through analysis of variance and means were compared using the Newman and Keuls test (Snedecor and Cochran, 1967). Visual rank data were processed using the Mann and Whitney test (Snedecor and Cochran, 1967).

RESULTS

After a period of active growth, with a mean growth rate (% per week) of 3.54, sexual maturation caused a decrease of growth in both sexes, down to a mean rate of 0.43. After spawning, the growth rate improved in females (1.53), while remaining at a significantly ($p < 0.05$) lower level in males (0.53).

The changes in muscle carotenoid concentrations associated with spawning and post-spawning recovery were different in male and female rainbow trout (*fig.* 1). Ten weeks before spawning, the major and highly significant ($p < 0.05$) effects were those of carotenoid supplementation to diet: muscle carotenoid content increased with increased carotenoid in the diet (A 100 *vs* A 50). At spawning, muscle carotenoid concentrations decreased in both sexes, and particularly in males. During the next four months, spawned females progressively recovered muscle carotenoid concentrations equivalent to those observed before spawning; in contrast, males recovered only one third of their initial muscle carotenoid concentrations. Compared to that large sexual difference, variation among groups fed supplemented diets appeared to be of little importance.

Average colour parameters (100 *a/b*) were calculated from the reflectance spectra of muscle of the individuals subjected for processing (*table* 1). Increasing muscle carotenoid concentrations caused significantly increased redness and gave a more reddish hue to the muscle. After spawning, redness decreased with decreasing pigment concentration. Muscles from astaxanthin fed fish (A 100) remained more red than

Table 1. – Colourimetric measurement (100 *a*/*b*) calculated from the reflectance spectra of rainbow trout muscle fed diets supplemented with synthetic carotenoids (mean ± standard error, $n = 5$).

	Males	Females
10 weeks before spawning		
CTL[1]	57.8 ± 3.0	53.2 ± 2.6
A 50	87.6 ± 6.0	101.0 ± 4.8
A 100	115.4 ± 6.1	119.6 ± 4.4
C 100	90.8 ± 4.1	94.0 ± 3.6
4 weeks after spawning		
CTL	58.8 ± 4.0	42.4 ± 3.8
A 50	59.6 ± 5.6	59.4 ± 9.6
A 100	74.0 ± 8.6	90.6 ± 11.3
C 100	71.6 ± 7.3	74.6 ± 10.5
8 weeks after spawning		
CTL	48.6 ± 5.0	40.6 ± 1.9
A 50	57.0 ± 5.5	86.2 ± 5.7
A 100	68.4 ± 5.1	78.2 ± 5.6
C 100	58.6 ± 3.6	68.6 ± 8.8
18 weeks after spawning		
CTL	49.8 ± 7.1	42.2 ± 4.4
A 50	70.8 ± 4.5	108.6 ± 4.4
A 100	84.4 ± 10.6	112.0 ± 4.0
C 100	62.6 ± 3.5	92.6 ± 3.0

([1]) CTL = control; A 50 and A 100 = control diet supplemented with astaxanthin 50 mg/kg and 100 mg/kg respectively; C 100 = control diet supplemented with 100 mg/kg canthaxanthin.

Table 2. – Visual rank evaluation of rainbow trout muscle fed diets supplemented with synthetic carotenoids (mean ± standard error, $n = 5$).

	Males	Females
10 weeks before spawning		
CTL[1]	4.0 ± 1.2	7.0 ± 1.2
A 50	17.2 ± 2.8	21.4 ± 3.9
A 100	30.2 ± 2.9	36.0 ± 1.4
C 100	26.4 ± 3.9	21.8 ± 2.5
Spawning		
CTL	9.4 ± 1.6	7.6 ± 2.9
A 50	16.6 ± 3.3	34.2 ± 2.2
A 100	25.6 ± 2.4	35.4 ± 1.9
C 100	17.0 ± 5.0	18.2 ± 4.1
4 weeks after spawning		
CTL	6.6 + 1.1	9.4 ± 3.6
A 50	13.2 ± 3.6	25.8 ± 3.0
A 100	19.0 ± 3.9	34.8 ± 2.2
C 100	23.4 ± 3.7	31.8 ± 3.4
8 weeks after spawning		
CTL	5.2 ± 1.2	6.2 ± 1.8
A 50	16.8 ± 1.4	33.6 ± 2.1
A 100	24.4 ± 2.0	33.6 ± 2.5
C 100	14.6 ± 2.1	29.6 ± 2.9
18 weeks after spawning		
CTL	4.4 ± 1.3	10.4 ± 1.9
A 50	17.6 ± 1.2	33.8 ± 2.5
A 100	17.6 ± 4.6	34.4 ± 2.7
C 100	15.8 ± 3.0	30.0 ± 0.7

([1]) CTL = control; A 50 and A 100 = control diet supplemented with astaxanthin 50 mg/kg and 100 mg/kg respectively; C 100 = control diet supplemented with 100 mg/kg canthaxanthin.

those from canthaxanthin fed fish (C 100). Moreover, females recovered their muscle colour within 18 weeks after spawning.

In the rank test, comparison of trout muscle with different astaxanthin or canthaxanthin concentrations revealed significant ($p < 0.05$) visual difference for males and females within sampling dates (*table* 2). Astaxanthin (A 100) caused higher rank values of the trout muscle than canthaxanthin at equal feed pigment concentration (C 100) until spawning.

Correlations analysis between the three methods of pigmentation of trout muscle measurement (*table* 3) showed significant within-sampling correlations between: 1) chemical analysis and colourimetric measurements ($r = 0.90$); 2) chemical analysis and visual rank evaluation ($r = 0.91$) and 3) colourimetric measurements and visual rank evaluation ($r = 0.86$). These correlations remained fairly constant throughout the experiment.

Table 3. – Correlation coefficients between the three methods of muscle pigmentation measurements used (calculated from the means for each sex in each carotenoid-supplemented batches, $n = 6$).

	P/C[1]	P/R	C/R
10 weeks before spawning	0.84*[2]	0.94**	0.86*
Spawning		0.90*	
4 weeks after spawning	0.89*	0.88*	0.74
8 weeks after spawning	0.91*	0.95**	0.94**
18 weeks after spawning	0.95**	0.98**	0.93**

([1]) P: Chemical analysis; C: colourimetric measurement; R: visual rank evaluation.
([2]) Significance level: * $p = 0.05$; ** $p = 0.01$.

DISCUSSION

Three different methods were used to appreciate the muscle pigmentation of trout: the chemical analysis and the colour measurement, which are two objective methods, and the rank test which is a subjective method. Sensory analyses have been performed on fish using different systems: the natural colour system (NCS) (SSI, 1979; Skrede and Storebakken, 1986); the Roche (Roche vitamins and fine chemicals division, Hoffmann-La Roche Inc., Nutley, USA) colour card for salmon (Skrede *et al.*, 1990; Smith *et al.*, 1992); or an arbitrarly flesh colour scale with values of 1 (no visual pigmentation) to 8 (maximum red) (Foss *et al.*, 1984; Aksnes *et al.*, 1986; Foss *et al.*, 1987; McCallum *et al.*, 1987; Sommer *et al.*, 1991). However, due to the inherent variability that occurs when measuring muscle pigmentation of fish using colour card (Skrede *et al.*, 1990; Smith *et al.*, 1992) the value of comparing results from one study with those of another is limited. Despite the influencing factors which control visual scoring, some observations are

worth mentioning. The rank test used in this experiment was the same as that used in poultry (Chemillier, 1977) and remains more precise than the other sensory evaluations, when colour differences are slight. However, this method does not allow comparison of samples collected at different times. In our experiment, the three methods (chemical analysis, colour measurement and rank test) were correlated satisfactorily within sampling operations. This is in agreement with previous findings showing relationship between sensory and instrumentally assessed colour in rainbow trout (Skrede *et al.*, 1990), instrumentally assessed colour and carotenoid concentration in rainbow trout (Choubert, 1982; Skrede *et al.*, 1990) or sensory and carotenoid concentration in coho salmon *Oncorhynchus kisutch* (Smith *et al.*, 1992).

A better deposition of astaxanthin over canthaxanthin in the muscle of salmonids has been reported (Foss *et al.*, 1984; Torrissen, 1986; Choubert and Storebakken, 1989; Torrissen , 1989; Bjerkeng *et al.*, 1990; No and Storebakken, 1992). In our experiment no significant differences were observed in pigment deposition in the muscle between fish fed synthetic astaxanthin and synthetic canthaxanthin. These contradictory results were similar to previous findings in rainbow trout and sea trout *Salmo trutta* (L.) (Foss *et al.*, 1987) or Atlantic salmon *Salmo salar* (L). (Storebakken *et al.*, 1987). The deposition mechanisms for the individual carotenoids in salmonids are not well known and are probably complex.

Carotenoid concentrations of rainbow trout muscle significantly decreased when fish became sexually mature (lowest concentrations were reached at spawning) despite the fact that fish were fed over this period. In the wild such a phenomenom is known since the fish feeding activity is reduced or even interrupted (anadromous migrating salmon): wild salmonids undergoing sexual maturation mobilize carotenoids from the flesh and selectively transfer them to the skin and gonads (Steven, 1949; Crozier, 1970; Shnarevitch and Sakhnenko, 1971; Kitahara, 1983; Ando, 1986). For farmed fish the pattern is somewhat different as fish are regularly fed even at a lower rate. It has been reported that the total amount of dietary canthaxanthin would be allocated about equally to muscle and ovary in rainbow trout (Choubert and Blanc, 1989) like astaxanthin in Atlantic salmon (Torrissen and Torrissen, 1985). Therefore the decrease in canthaxanthin concentration in female muscle was in contrast with previous findings (Choubert and Blanc, 1989) and may be due to the lower dietary canthaxanthin level (100 *vs* 200 canthaxanthin/kg diet). The decrease of growth rate in both sexes at spawning would also explain this finding. However, this does not appear enough, by itself, to explain the depigmentation since, in immature trout, the depletion of canthaxanthin during starvation was found to be a very slow process (Choubert, 1985). Another explanation would be the increasing metabolism of carotenoids to vitamin A in the mature fish (Al-Khalifa and Simpson, 1988; Guillou *et al.*, 1989) but it concerns only astaxanthin since no conversion from canthaxanthin to vitamin A has been reported.

Females recovered their muscle carotenoid concentrations after spawning. In males, the recovery was slower, which might be explained by the fact that males are mature earlier and remain so over a longer period than the females (Sano, 1960; Aksness *et al.*, 1986). This is corroborated by comparison of the growth rates in the present experiment.

In usual fish farming practice, sexually mature individuals are not marketed, since a significant alteration of the flesh composition and concomitant deterioration of the flesh quality occur (Torrissen and Torrissen, 1985; Aksnes *et al.*, 1986). The use of high carotenoid levels (as high as 100 mg carotenoid/kg diet) in the diet of rainbow trout prior to spawning did not prevent the depletion of carotenoids from the flesh, as was reported for Atlantic salmon (Helland *et al.*, 1990). Therefore, the fish producer should avoid providing dietary carotenoids to fish, at least to males, in this biological condition.

Acknowledgements

The authors wish to thank Laurence Larroquet, F. Vallée and Jacqueline Vasseur (Produits Roche s.a., 59, rue Pasteur, 94120 Fontenay-sous-Bois, France) for their technical assistance and L. Casenave for maintenance of the experimental animals. The authors thank also J. Chemillier (Produits Roche s.a., 52, bd du Parc, 92521 Neuilly-sur-Seine cedex, France), J.-P. Belliot (Produits Roche s.a., 59, rue Pasteur, 94120 Fontenay-sous-Bois, France), K. Streiff and T. Latscha (F. Hoffman-La Roche & co, 4002 Basel, Switzerland) for their interest.

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