Pigmenting efficacy of astaxanthin fed to rainbow trout *Oncorhynchus mykiss*: Effect of dietary astaxanthin and lipid sources

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Abstract

The aim of the present experiment was to investigate the effect of two different dietary types of oil (fish oil (FI) and olive oil (OL)) on the pigmenting efficacy of astaxanthin from the green micro-algae *Haematococcus pluvialis* (ALG) (total amount of carotenoid pigments 32 mg kg^{-1} on a dry weight basis of which astaxanthin accounted for 98.6%) and from the synthetic astaxanthin (AST) in terms of astaxanthin serum concentration, induced muscle colour, and astaxanthin muscle retention in rainbow trout for 6 weeks. Diets with different oil sources were well accepted by fish. At the end of the experiment there were no significant differences between fish fed different diets in final mean weight, specific growth rate, or feed conversion ratio. Fish fed AST showed higher ($P<0.05$) serum concentrations than those fed ALG. Moreover fish fed diets OL displayed higher ($P<0.05$) serum astaxanthin levels than those fed diets FI. Fish muscle colour parameters reacted differently according to fish feed. Over 6 weeks of feeding L^* compared to L^* of initial sampling time showed a decrease more marked for trout fed ASTFI than ALGFI. Fish fed ASTOL and ALGOL displayed intermediary values. On the contrary the other colour parameters increased except hue $H(°)_{ab}$ which did not show any change whatever diet was fed to the fish. Chroma C^*, a^*, and b^* data obtained for fish fed AST were higher than those obtained for fish fed ALG. Muscle astaxanthin concentrations were lower ($P<0.05$) for fish fed algae than for those fed synthetic astaxanthin. After 6 weeks of experiment muscle astaxanthin levels were not different ($P>0.05$) for fish fed olive or fish oil. Muscle astaxanthin retention was higher ($P<0.05$) for fish fed AST than for fish fed ALG.

Introduction

The colour of salmonids is an important criterion of quality. The typical red to pink muscle colour of salmonids is due to astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione), a carotenoid from dietary origin that fish cannot synthesize. In the wild fish derive astaxanthin through their prey organisms while in intensive rearing system astaxanthin is added to the feed in the form of nature identical synthetic beadlets (Bjerkeng et al., 1999). However, more and more natural alternative sources are expected to be used as for example the green micro-algae *Haematococcus pluvialis*. This algae, when

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subjected to stress full culture conditions, produces astaxanthin as primary carotenoid up to 1–3% of dry weight (Lorenz and Cysewski, 2000).

Carotenoids are lipid-soluble compounds. Therefore the amount and type of lipid with which they are consumed may influence their absorption. Several studies involving different amounts of dietary lipid have produced conflicting results (see review by Torrissen et al., 1989). On the other hand information on the effect of different types of dietary lipid on carotenoid utilization is relatively sparse. Atlantic salmon fed diets containing menhaden oil had higher canthaxanthin in their muscle than salmon fed diets containing soybean oil (Hardy et al., 1987; Regost et al., 2004) or rapeseed oil (Regost et al., 2004). However, no difference in astaxanthin concentration was noted in the muscle of salmon when fish oil was replaced by rapeseed oil (Bell et al., 2001) or palm oil (Bell et al., 2002). On the other hand it has been shown that salmon fed high $n-3$ PUFA (polyunsaturated fatty acids) oil had higher muscle astaxanthin content than those fed herring oil (Bjerkeng et al., 1999).

The aim of the present experiment was to investigate the effect of different dietary oils on the pigmenting efficacy of astaxanthin in terms of astaxanthin serum concentration, induced muscle colour, and astaxanthin muscle retention by feeding olive oil replacing fish oil to rainbow trout for 6 weeks. Astaxanthin sources were green micro-algae *H. pluvialis* and synthetic astaxanthin.

Materials and methods

Fish and feeding management

The experiment took place at the INRA Experimental Station of Donzacq (Landes Department, South West of France). Two hundred and seventy six rainbow trout, *Oncorhynchus mykiss*, from the same parental stock were randomly divided in four groups in square tanks (300 l) set in parallel rows supplied with spring water (constant temperature 17±1 °C; pH 7.4; Cl^- 22.5 mg l^{-1}; Ca^{2+} 75 mg l^{-1}; dissolved oxygen 8 mg l^{-1}) at a rate of 1.4 l min^{-1}. Tanks received a natural photoperiod (October to November). Prior to introduction to the experimental diets and during the acclimatization period, fish were fed a commercial non-astaxanthin supplemented feed for 15 days (Trouw France, Fontaine-les-Vervins, France). Then fish, reaching a mean weight of 185 g, were fed the 4 experimental diets in triplicate (23 fish $tank^{-1}$) for 6 weeks. Fish were hand fed twice a day (08:30 h, 16:30 h) an equal quantity of feed 7 days $week^{-1}$ at a rate of 2.5% body weight day^{-1} (BWD) and complete feed ingestion was assessed visually. At each 2-week period an adjustment of the daily ration was made according to the fish growth.

Experimental diets

Four test diets with the same basal composition (Table 1) were supplemented with two different lipid sources, fish oil and olive oil, compositions of which are given in Table 2 and two different astaxanthin sources at

Table 1
Formulas, ingredients and chemical composition of the 4 experimental feeds (AST, ALG: diets containing synthetic astaxanthin or algae, respectively; FI, OL: diets containing fish oil or olive oil, respectively)

Diet label	ASTFI	ASTOL	ALGFI	ALGOL
Feed ingredients (g kg^{-1})				
Fish meal [a]	580	580	580	580
Gelatinised wheat starch [b]	160	160	160	160
Crude wheat starch [c]	130	130	130	130
Olive oil [d]	–	10	–	10
Fish oil [e]	10	–	10	–
Vitamin mix [f]	10	10	10	10
Mineral mix [g]	10	10	10	10
Sodium alginate [h]	10	10	10	10
Astaxanthin [i]	+	+		
Algae			+	+
Feed chemical composition [j]				
Dry matter DM (%)	95.38	94.42	94.91	94.58
Total lipids (% DM)	15.24	15.26	15.01	16.15
Astaxanthin (mg kg^{-1} DM)	80.2	78.1	72.6	71.1

[a] Norwegian herring meal, Norse LT94. Sopropêche, Boulogne-sur-mer, France.
[b] Amidex, Ogilvie Aquitaine, Bordeaux, France.
[c] Amidaine B, Ogilvie Aquitaine, Bordeaux, France.
[d] Olive oil extravirgin quality, Euroliva Cia Oleícola S.L., Plasencia, Spain.
[e] Feedoil, Scandinavian fish oil. Sopropêche, Lorient, France.
[f] INRA 762. Vitamin mix contained the following mixed with cellulose (g kg^{-1} mix): vitamin A (500,000 IU g^{-1}), 1.5; vitamin D3 (100,000 IU g^{-1}), 1.5; vitamin E (500 IU g^{-1}), 6; vitamin K, 0.25; thiamin, 0.75; riboflavin, 1.5; pyridoxine, 0.75; nicotinic acid, 8.75; vitamin C, 25; folic acid, 0.25; vitamin B_{12} (1000 mg kg^{-1}), 2.5; inositol, 50; biotin (2 mg kg^{-1}), 6.25; calcium pantothenate, 2.5; choline (50 mg kg^{-1}), 200.
[g] INRA 763. Mineral mix contained the following ingredients (g kg^{-1} mix): calcium carbonate, 215; magnesium hydroxide, 124; KCl, 90; ferric citrate, 20; KI, 0.4; NaCl, 40; calcium hydrogen phosphate ($CaHPO_4$), 500; copper sulfate, 3; zinc sulfate, 4; cobalt sulfate, 0.2; manganese sulfate, 3.
[h] Alginate GF 150. Louis François Exploitation, Saint Maur, France.
[i] CAROPHYLL® pink, DSM (formerly F. Hoffman-La Roche), Basel, Switzerland.
[j] Means of 2 independent determinations.

Table 2
Fatty acid composition of dietary oils (% by wt of total fatty acids)[1]

Fatty acid	Fish oil	Olive oil
12:0	0.2	0
14:0	15.4	0
14:1	0.2	0
15:0	0.7	0
16:0	19.7	14.3
16:1	9.4	1.3
16:2*n*−7	0.4	0
16:2*n*−4	1.0	0
16:4*n*−1	0.3	0
17:0	0.2	0
17:1	0.3	0
18:0	1.3	0
18:1	16.1	75.2
18:2*n*−6	1.8	4.5
18:3	0.3	0
18:3*n*−3	1.1	0.6
18:4	3.1	0
20:0	0.1	3.4
20:1	8.1	0.2
20:2	0.1	0
20:4	0.2	0
20:5	4.7	0
22:1	7.4	0
22:5	0.2	0
22:6*n*−3	3.5	0
SFA[2]	37.6	17.7
MUFA[3]	41.5	76.7
PUFA*n*−6[4]	4.1	4.5
PUFA*n*−3	13.2	0.6
SFA/PUFA	2.2	3.5
PUFA*n*−3/*n*−6	3.2	0.1

[1]Mean of duplicate samples; [2]Saturated fatty acids; [3]Monounsaturated fatty acids; [4]Polyunsaturated fatty acids.

a level of 75 mg astaxanthin kg^{-1} of feed. The two carotenoid sources used were algae *H. pluvialis* strain CCAP-34/7 (Culture Collection of Algae and Protozoa, Windermere, UK) cultivated under the same conditions as described by Mendes-Pinto et al. (2001), and commercial beadlets of 8% (w/w) astaxanthin content (CAROPHYLL® Pink, DSM Nutritional product (formerly F. Hoffmann-La Roche), Basel, Switzerland). Algae were mechanically ground in a ball grinder (grinder Dangoumeau, Prolabo, Fontenay-sous-bois, France) for 5 min prior to diet incorporation and the cell wall disruption was assessed by optical microscopy (Bio-Med, Leitz, Westlar, Germany). Diets were pelleted using a steamless pelleting machine (M-Labor, Simon Heesen B.V., Boxtel, The Netherlands) through a 4.5 mm dye. Pellets were allowed to dry at 38 °C for 4 h (dryer Bulkit, Monclar, France) and were stored at 4 °C prior to use. Proximate composition of experimental diets is given in Table 1.

Sampling and analytical methods

Four fish per tank were sampled and sacrificed by a sharp blow on cervical vertebrae at regular intervals of 2 weeks (0, 2, 4, and 6 weeks). Fish blood was collected from the caudal artery with a 2 ml non-heparinized disposable syringes fitted with 0.6×25 mm disposable needles (Becton Dickinson France, Le Pont de Claix, France) and analysed for astaxanthin as described by Mendes-Pinto et al. (2004).

Muscle colour was assessed immediately post-slaughter by using a chromameter (mod. CR200, Minolta Co. Ltd., Osaka, Japan) equipped with a 8 mm diameter aperture and calibrated on a white reference plate before use as described by Choubert et al. (1997). Measurements were processed at three locations along the fillet above the lateral line: close to the head; midway between the head and the tail; and close to the tail. All measurements were performed in the colorimetric space $L^* a^* b^*$ in accordance with the recommendations of the Commission Internationale de l'Éclairage (CIE, 1976). They were then transformed in the $L^* C^* H(°)_{ab}$ colorimetric space according to Wyszecki and Stiles (1967). In this space the three dimensional characteristics of colour appearance are the lightness attribute L^* and the two chromatic attributes hue $H(°)_{ab}$ and chroma C^*.

Biochemical analyses of diets and muscles were dry matter (DM), total lipids (Folch et al., 1957) and astaxanthin concentrations (Choubert and Storebakken, 1989). For carotenoid extraction, micro-algal biomass was prior mechanically ground for 5 min (grinder Dangoumau, Prolabo, Fontenay-sous-Bois, France). Determination of total carotenoids content was made by UV–Visible spectrophotometry according to Mendes-Pinto et al. (2004). For diets containing algae, astaxanthin was analysed after lipid extraction (Folch et al., 1957) and quantified by spectrophotometry as described above. For diets

Table 3
Serum astaxanthin concentrations[1][2] of trout fed different astaxanthin and lipid sources calculated as ratio [total blood astaxanthin per unit body weight to cumulative astaxanthin intake per unit body weight]

Sampling time	Diet[3]			
	ASTFI	ASTOL	ALGFI	ALGOL
2 weeks	12.1±3.7^{a,b}	15.7±5.4^{a}	9.1±3.7^{a,b}	6.9±2.0^{b,c}
4 weeks	33.9±7.7^{b}	69.5±23.0^{a}	7.0±2.3^{c}	9.0±2.8^{c}
6 weeks	17.9±4.9^{b}	48.6±20.1^{a}	38.3±17.5^{a}	21.9±3.0^{b}

[1]Mean±S. D., $n=4$.
[2]Within a row means with different superscripts are significantly different, Tuckey's multiple comparisons test, ($P<0.05$).
[3]AST, ALG: diets containing synthetic astaxanthin or algae, respectively; FI, OL: diets containing fish oil or olive oil, respectively.

Table 4
Colour parameters of trout muscle during two, four, six weeks (2 w, 4 w, and 6 w, respectively) sampling times[1][2]

Colour parameters[4]	Sampling times	Diets[3]			
		ASTFI[3]	ASTOL	ALGFI	ALGOL
L^*	0	41.32±1.76	41.32±1.76	41.32±1.76	41.32±1.76
	2 w	40.98±1.44^{b}	40.41±1.59^{b}	42.76±2.08^{a}	41.82±0.85^{a,b}
	4 w	39.61±1.15^{b}	38.48±1.94^{b}	41.65±1.34^{a}	40.00±2.36^{a,b}
	6 w	37.98±2.31^{c}	38.50±1.58^{b,c}	41.33±2.36^{a}	40.16±1.55^{a,b}
C^*	0	2.48±0.45	2.48±0.45	2.48±0.45	2.48±0.45
	2 w	6.85±2.28^{a}	7.01±2.55^{a}	2.94±1.82^{b}	3.82±1.35^{b}
	4 w	9.40±3.04^{a,b}	11.31±2.62^{a}	5.23±2.62^{c}	7.36±2.24^{b,c}
	6 w	13.17±3.46^{a}	12.79±1.87^{a}	7.13±2.73^{b}	8.63±1.62^{b}
$H(°)_{ab}$	0	154.89±91.76	154.89±91.76	154.89±91.76	154.89±91.76
	2 w	56.32±7.21^{a,b}	57.38±4.81^{a,b}	73.52±30.88^{a}	54.54±11.22^{b}
	4 w	59.28±7.85^{a}	58.04±2.69^{a}	57.71±8.08^{a}	56.15±5.84^{a}
	6 w	56.54±3.09^{a}	58.63±2.88^{a}	55.77±4.89^{a}	57.55±3.06^{a}
a^*	0	0.94±0.46	0.94±0.46	0.94±0.46	0.94±0.46
	2 w	3.66±1.16^{a}	3.72±1.74^{a}	1.44±0.68^{b}	2.06±0.64^{b}
	4 w	5.18±1.44^{a,b}	6.05±1.73^{a}	2.61±1.16^{c}	3.99±1.19^{b,c}
	6 w	7.21±1.89^{a}	6.67±1.05^{a}	3.95±1.59^{b}	4.65±1.03^{b}
b^*	0	1.69±0.84	1.69±0.84	1.69±0.84	1.69±0.84
	2 w	5.75±2.04^{a}	5.91±2.24^{a}	2.40±1.85^{b}	3.13±1.33^{b}
	4 w	7.73±2.98^{a,b}	9.54±2.03^{a}	4.47±2.43^{c}	6.13±2.01^{b,c}
	6 w	10.99±2.96^{a}	10.89±1.67^{a}	5.88±2.29^{b}	7.25±1.33^{b}

[1]Data are mean±SD, $n=4$.
[2]Means on the same line not sharing the same letter are significantly different ($P<0.05$).
[3]AST, ALG: diets containing synthetic astaxanthin or algae, respectively; FI, OL: diets containing fish oil or olive oil, respectively.
[4]L^* = lightness with 100 = absolute white and 0 = absolute black; C^* = chroma calculated as $(a^*+b^*)^{1/2}$ (Wyszecki and Stiles, 1967); $H(°)_{ab}$ = Hue angle determined as arctan b^*/a^* (Wyszecki and Stiles, 1967); a^* = "redness" coordinate; b^* = "yellowness" coordinate.

containing synthetic astaxanthin the method of Schüep and Schierle (1995) was performed.

Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids using boron trifluoride methanol according to Santha and Ackman (1990). The chromatograph (mod. 3400, Varian inc., Palo Alto, USA) was equipped with a DB Wax fused silica capillary column (30 m×0.25 mm i.d., film thickness: 0.25 μm, J and W Scientific, Folsom, USA). Helium was used as carrier gas (1.4 ml min^{-1}) and the thermal gradient was 100 to 180 °C at 8 °C min^{-1}, 180 to 220 °C at 4 °C min^{-1} and a constant temperature of 220 °C during 25 min. Injector and flame ionisation detector temperatures were 260 and 250 °C, respectively. Fatty acid methyl esters were identified and quantified by comparison with known standard mixtures (# 189-19, Sigma-Aldrich Co, Saint Quentin Fallavier, France).

Serum astaxanthin concentrations were calculated as ratio [total blood astaxanthin per unit body weight to cumulative astaxanthin intake per unit body weight]. A mean whole trout blood volume of 3% BW at a water temperature of 16 °C was used for calculation according to Nikinmaa et al. (1981).

Trout muscle astaxanthin retention was calculated according to the following equation (Choubert and Luquet, 1982):

Muscle astaxanthin retention
= [final muscle astaxanthin concentration (mg kg^{-1} DM)
× (final muscle mean weight (kg DM))
−initial muscle astaxanthin concentration (mg kg^{-1} DM)
× (initial muscle mean weight (kg DM))]/
[feed consumed (kg DM)
× diet astaxanthin concentration (mg kg^{-1} DM)]100.

Data were subjected to analysis of variance and Tuckey's multiple comparison test using SAS-GLM procedures (SAS, 1989). A significance level of 5% was assumed.

Results

Fish oil (Table 2) contained 37.6% saturated fatty acids, mainly 14:0 (15.4%) and 16:0 (19.7%), 41% monounsaturated fatty acids, mainly 18:1 (16.1%) and 20:1 (8.1%), and 17.3% polyunsaturated fatty acids, mainly 18:3 $n-3$ (1.1%), 18:2 $n-6$ (1.8%), and 22:6 n

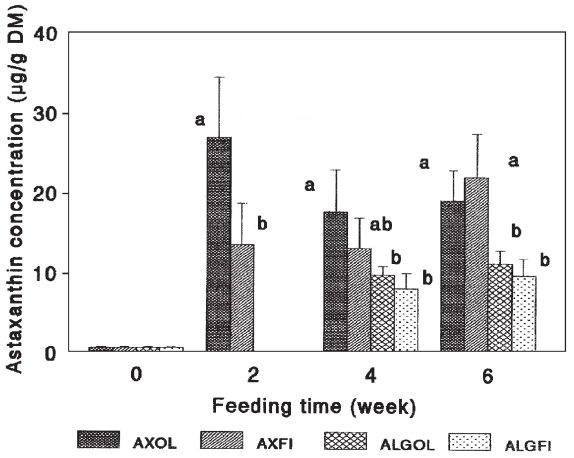

Fig. 1. Muscle astaxanthin concentrations of fish fed during 6 weeks diets containing synthetic astaxanthin (AST) or algae (ALG), respectively; and containing fish oil (FI) or olive (OL) oil, respectively.

−3 (3.5%). Olive oil contained 17.7% saturated fatty acids, mainly 16:0 (14.3%) and 20:0 (3.4%), 76.7% monounsaturated fatty acids, mainly 18:1 (75.2%), and 5.1% polyunsaturated fatty acids, mainly 18:2 $n-6$ (4.5%).

The total amount of carotenoid pigments in microalgae *H. pluvialis* was 32 mg kg^{-1} on a dry weight basis of which astaxanthin accounted for 98.6%.

Diets with different oil sources were well accepted by fish and no mortalities occurred. At the end of the experiment there were no significant ($P>0.05$) differences between fish fed the different diets in final mean weight (overall mean, 345 g), specific growth rate (overall mean, 0.96) and feed conversion ratio (overall mean, 1.66).

Serum astaxanthin concentrations of trout fed different astaxanthin and lipid sources are reported in Table 3. Fish fed AST showed higher ($P<0.05$) astaxanthin serum concentrations than those fed ALG. Moreover fish fed diets containing OL displayed higher ($P<0.05$) serum astaxanthin levels than those fed diets containing FI. Fish fed diet ASTOL showed highest levels of serum astaxanthin while fish fed ALGOL presented the lowest levels of serum astaxanthin except at the 6 week sampling time. A positive time effect was also only noted for astaxanthin serum concentration from fish fed algae.

Colour parameters of muscle of trout fed the different experimental diets are shown in Table 4. Over 6 weeks of feeding L^* compared to L^* of initial sampling time showed a decrease more marked for trout fed ASTFI than ALGFI. Fish fed ASTOL and ALGOL displayed intermediary values. On the contrary the other colour parameters increased except hue $H(°)_{ab}$ which did not show any difference among dietary treatment groups. Chroma C^*, a^*, and b^* data obtained for fish fed AST were higher than those obtained for fish fed ALG. Hues were similar among fish fed the different diets and L^* showed lower values for fish fed AST compared to fish fed ALG.

Muscle astaxanthin concentrations are plotted on Fig. 1. Muscle astaxanthin concentrations were lower ($P<0.05$) for fish fed algae than for those fed synthetic astaxanthin. After 6 weeks of experiment muscle astaxanthin levels were not different ($P>0.05$) for fish fed olive or fish oil.

Astaxanthin retentions in the muscle of rainbow trout fed the different diets are given in Table 5. At the end of the first two weeks of experiment fish receiving ASTOL displayed a significant ($P<0.05$) higher muscle astaxanthin retention than fish fed ASTFI. Muscle astaxanthin retentions calculated after 4 weeks or 6 weeks were lower than those calculated after 2 weeks. Muscle astaxanthin retentions were higher ($P<0.05$) for fish fed synthetic astaxanthin than for those fed algae. There were no (pigment source × oil source) or (sampling time (pigment source × oil source)) interactions.

Discussion

The oil sources used in this experiment were chosen because they have different physical properties. Fish oil has higher levels of saturated fatty acids and higher $n-3$ polyunsaturated fatty (PUFA) acids than olive oil while

Table 5
Astaxanthin retention in the muscle of rainbow trout fed the different diets [(1)(2)(3)]

Parameter	Periods	Treatments			
		ASTFI	ASTOL	ALGFI	ALGOL
Astaxanthin retention	0–2 w	4.42 ± 3.36^{b}	8.36 ± 2.80^{a}	n.a.[(4)]	n.a.
	0–4 w	$2.43\pm2.12^{a,b}$	5.65 ± 3.85^{a}	1.26 ± 0.31^{b}	$1.87\pm0.81^{a,b}$
	0–6 w	5.97 ± 2.03^{a}	5.28 ± 1.43^{a}	1.80 ± 1.09^{b}	2.79 ± 0.78^{b}

[(1)]Data are mean±SD, $n=4$.
[(2)]Means on the same line not sharing the same letter are significantly different ($P<0.05$).
[(3)]AST, ALG: diets containing synthetic astaxanthin or algae, respectively; FI, OL: diets containing fish oil or olive oil, respectively.
[(4)]n.a.: not available.

olive oil (extra virgin quality and cold extracted) had higher levels of monounsaturated fatty acids than fish oil. The fish oil used was not attributed to one particular fish species but to Scandinavian fish as specified by the manufacturer. Fatty acid contents were in the range of fatty acid contents reported for other fish oils (Hardy et al., 1987; Christiansen et al., 1993; Bjerkeng et al., 1999) with a PUFA content similar to herring oil PUFA content (medium PUFA).

The strain of *H. pluvialis* used in this experiment has been reported in previous studies to contain astaxanthin as the major carotenoid pigment in an amount as high as 98.6% of total carotenoids and to be efficient in terms of trout flesh pigmentation (Barbosa et al., 1999; Mendes-Pinto et al., 2004). As algae occur in an encysted form surrounded by a thick cell wall which may prevent astaxanthin absorption by fish (Choubert and Henrich, 1993), algae biomass was carefully ground before being added to the diets. However, this grinding step may explain some differences observed in astaxanthin concentrations between different series of test diets.

The addition of vegetable oil as olive oil up to 10% in the diet of rainbow trout did not affect growth or derived parameters such as specific growth rates or feed conversion ratio. This is in agreement with previous reports for salmon receiving in their diet vegetable oils such as soybean oil (Hardy et al., 1987; Thomassen and Røsjø, 1989; Regost et al., 2004), rapeseed oil (Thomassen and Røsjø, 1989; Regost et al., 2004; Torstensen et al., 2004) or olive oil (Torstensen et al., 2004), or for European sea bass receiving in their diet rapeseed or olive oil (Mourente et al., 2005).

As there was a difference in amounts of astaxanthin in the test diets, serum astaxanthin concentrations of trout fed different diets were calculated as ratio [total blood astaxanthin per unit body weight to cumulative astaxanthin intake per unit body weight]. In this case our results showed that fish fed diets containing synthetic astaxanthin displayed higher serum concentration than those fed astaxanthin from this strain of algae. This is in agreement with our earlier work (Mendes-Pinto et al., 2004).

Our result also showed that fish fed diets containing olive oil displayed higher serum astaxanthin levels than those fed diets containing fish oil. Decreasing carotenoid absorption when dispersed in PUFA was reported in rat (Clark et al., 2000). Moreover, in the rat, dietary lipids of plant origin, in contrast to those of animal origin, promote the accumulation of β-carotene in plasma (Alam et al., 1989) by influencing chilomicron metabolism (Lambert et al., 1998). The reason for this is not fully understood. However, results in salmon seem contradictory as blood plasma astaxanthin was unaffected by high PUFA dietary oil (Bjerkeng et al., 1999). An explanatory for this discrepancy would be that oils contain different amounts of stanol and sterols which interfere with the absorption of carotenoids (Nguyen, 1999).

In our experiment fish muscle colour parameters reacted differently according to fish feed. Hue ($H(^{\circ})_{ab}$) was not affected by diets. Lightness (L^*) decreased while chroma (C^*), a^* and b^* increased over time as fish consumed pigmented diets. This is in agreement with previous findings (Choubert, 1982). Muscle colour attributes were more affected by astaxanthin source than by oil source. Fish fed naturally identical synthetic astaxanthin showed higher chroma, a^* and b^* than fish fed algae. The lower efficacy of algae *H. pluvialis* was reported previously for rainbow trout (Sommer et al., 1991; Choubert and Henrich, 1993; Choubert et al., 1995) and was explained as probably due at least in part to the high amount of esterified carotenoid (Renstrøm et al., 1981) which has been found to be less efficiently used by salmonids (Storebakken et al., 1987).

Reports of dietary oil source effects on colour parameters of fish muscle have been contradictory and are not yet understood. Variations in colour parameters of fish muscle fed vegetable oils are also confusing; no variation in a^* for salmon fed soybean oil was reported (Thomassen and Røsjø, 1989) while a decrease in a^* was noted in salmon muscle fed rapeseed oil (Thomassen and Røsjø, 1989; Regost et al., 2004) or soybean oil (Regost et al., 2004), fish muscle being less red than that of fish fed fish oil diets.

Muscle astaxanthin retention was higher for fish fed synthetic astaxanthin than for fish fed algae. Although astaxanthin retention data are sparse in the literature retention coefficients noted in our experiment were of the same order than those reported earlier (Choubert and Henrich, 1993; Choubert et al., 1995) for other algae strains. According to Choubert et al. (1995) this low retention is likely due to the low digestibility of algae astaxanthin.

Fish receiving the diet with synthetic astaxanthin plus olive oil showed a higher muscle astaxanthin retention than fish fed the diet with synthetic astaxanthin plus fish oil. The only main reason which may be put forward is the difference in fatty acid composition which may affect i) astaxanthin absorption across the gastrointestinal tract of fish, or ii) molecular processes involved in chylomicron turn-over as noted for β-carotene in women fed a meal rich in sunflower oil (Hu et al., 2000). However, in salmon fed diets with capelin or high PUFA oil, similar muscle astaxanthin retentions were observed (Bjerkeng et al., 1999) which are not yet explained.

Conclusion

One interesting observation in this study was that olive oil can be used up to 10% replacement of dietary fish oil for rainbow trout during the grow out period (6 weeks) without negative effects on mortality or growth. For astaxanthin content in serum and muscle of trout the effects of dietary oil source gave faint results. On one hand, fish fed diets OL displayed higher ($P<0.05$) serum astaxanthin levels than those fed diets FI. On the other hand after 6 weeks of experiment muscle astaxanthin levels were not different ($P>0.05$) for fish fed olive or fish oil. The similar retention of astaxanthin in trout fed diets with FI and OL oils cannot be explained at present and further investigations are necessary. Moreover organoleptic study should be made to find changes, if any, in quality parameters of fish muscle.

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