

PAPER

Effects of dietary supplementation with krill meal on pigmentation and quality of flesh of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Effects of administration of krill meal and synthetic astaxanthin during the finisher phase of the fattening cycle of rainbow trout on flesh pigmentation and quality traits were studied. The inclusion of krill meal increased the body weight and size and decreased the peri-visceral fat and visceral weight indices. The astaxanthin diet produced the highest accumulation of total carotenoids in the fillet compared to the krill meal diet: the difference was significant after 15 days of feeding (2.50 vs 2.10 mg/kg) till the end of the trial (5.00 vs 4.80 mg/kg). The same pattern was observed for astaxanthin concentration with the highest values in the fillets of fish fed the astaxanthin diet. Fillet lightness (L*) was not affected by trout diets whereas redness (a*) and yellowness (b*) were significantly higher in fish fed the astaxanthin diet until day 30 of the trial. Hue was not affected by feeding, whereas chroma was significantly higher in the fish fed astaxanthin throughout the trial except on day 45 of sampling. Trout fed the krill meal diet had a paler pink-red colour on the SalmoFan scale than those receiving the astaxanthin diet. No significant differences emerged in proximate composition and cholesterol content of trout in the two groups. The fatty acid profile of the fillets reflected the fatty acids of the diets administered to the trout: eicosapentaenoic, docosahexaenoic and docosapentaenoic acids and total n-3 polyunsaturated fatty acids were significantly higher in the fish fed the krill meal.

Introduction

The concentration of carotenoid pigments in salmonid muscles has been studied for many years with the aim of improving the efficiency of the pink-red colouration of the flesh and comparing the colour between wild and farmed salmon (Storebakken *et al.*, 1987; Torrissen, 1989; Tibaldi and Ballestrazzi, 1990; Torrissen *et al.*, 1995; Nickell and Springate, 2001). In fact, in farmed salmonids, flesh colour is the most important quality trait together with freshness and nutritional value of the product (Anderson, 2000). Synthetic astaxanthin and canthaxanthin are still the most important sources of pigments used in the feeding of farmed salmonids because of their high pigmentation ability of both skin and flesh. However, in the last few years the use of synthetic pigments has been discussed in relation to the possible damage to human health deriving from the consumption of fish rich in these synthetic colouring additives (Anderson *et al.*, 1997; Georgakopoulos and Thomson, 2005). In a review on carotenoid deposition and pigmentation regimens (Nickell and Springate, 2001), 40-60 mg/kg astaxanthin proved the most efficient dietary dose for optimal pigment retention in Atlantic salmon while in freshwater rainbow trout the level ranged from 50 to 70 mg/kg astaxanthin.

Although the European Food Safety Authority has indicated that the current approved practice of feeding canthaxanthin to layers, meat-type chickens and salmonids does not raise safety concerns for the consumer (EFSA, 2007), many farmers have decided to adopt guidelines that forbid the use of synthetic pigments in feeds. Moreover, the law currently in force regarding organic fish products (EC Regulation No. 710/2009) only recognises the use of astaxanthin as a natural pigment in the feeds to colour fish muscle instead of synthetic additives. In this situation, studies on natural sources of astaxanthin able to favour flesh pigmentation for an alternative pigmentation of salmonids have increased. Pigments such as shrimp waste (Diler and Gökoğlu, 2004), red yeast *Phaffia rhodozyma* (Gentles and Haard, 1990; Bjerkeng *et al.*, 2007), green algae *Haematococcus pluvialis* (Choubert and Heinrich, 1993) and red pepper and marigold flower (Yanar *et al.*, 2007) have been tested with different results. The use of krill, including around 85 species of pelagic crustaceans belonging to the order *Euphausiacea*, in feeds to colour different salmon species has also been investigated (Storebakken 1988; Scott *et al.*, 1994), in addition to considering the possibility of using krill

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meal to replace fish meal (Moren *et al.*, 2006; Yoshitomi *et al.*, 2007). Analysing the nutritional value of frozen Antarctic krill, Gliotti *et al.* (2008) found a content of approximately 78% protein and 8% fat, whereas n-3 polyunsaturated fatty acids (PUFA) amounted to 27% of the total fatty acids. In overweight and obese men and women, krill supplementation increased plasma concentrations of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, without adverse effects on tolerability and safety when it was compared with menhaden and olive oil (Maki *et al.*, 2009). The purpose of our study was to investigate the effect of the dietary inclusion of krill meal, during the finisher phase of the fattening cycle, on the fillet pigmentation of rainbow trout. Fillet colour, astaxanthin and carotenoid concentrations, growth performance and flesh quality traits, such as proximate composition, fatty acid profile and cholesterol content, were compared with those of trout receiving a commercial feed containing synthetic astaxanthin.

Materials and methods

The trial was performed at a trout farm located in Sefro (Italian Central Apennine). A total of 4400 rainbow trouts (*Oncorhynchus mykiss*), weighing 453±57 g (12 months old) and measuring 34±2 cm, were stocked in two 100-m³ race-

ways at the same stocking density (22 fish/m³). The trial lasted 60 days during which the main physicochemical water parameters were monitored regularly and nitrogen and phosphorous compounds analysed at the laboratory following APHA standard methods (APHA, 1989).

Feeds and feeding management

Two isonitrogenous diets were used. The experimental diet (KR), formulated with GMO-free certified ingredients, included krill meal obtained from the *Euphasia superba* species as the protein and pigment source. The chemical composition of this ingredient is reported in Table 1. The fluoride content in the krill meal (130 mg/kg) was under the maximum limits (150 mg/kg feed) set by Annex I to Commission Directive 2003/100/EC. Commercial feed (AS) was based on soybean extracted meal, fish meal and wheat meal as the main ingredients and contained astaxanthin (90 mg/kg). The feed-stuffs, the proximate composition and the fatty acid profile of the two diets are reported in Tables 2 and 3. The feeds were administered to the trouts at the daily rate of 1% body weight, according to water temperature and fish weight.

Colour measurements, carotenoid and astaxanthin content

Every 15 days, 10 fish per group were sacrificed for the colour measurements and pigment content of the flesh, whereas the proximate composition and lipid profile of the fillet were determined at the end of the trial on 10 trouts per group. Immediately post-slaughter, muscle colour was assessed by a reflectance colourimeter (Minolta mod. Chroma Meter II CR-300, Minolta Italia SPA, MI, Italy) using the illuminant source D65 and 10° standard observer, set on multimeasure mode. Measurements were carried out according to the recommendations of the International Commission on Illumination (CIE, 1978), reporting values of lightness (L*; negative value: blackness, positive value: whiteness), redness (a*; negative: greenness, positive: redness) and yellowness (b*; negative: blueness, positive: yellowness). Readings were obtained at four positions along the left fillet, of which two were carried out above the lateral line and the other two below the lateral line. The averages of the three measurements were calculated to determine the L*, a* and b* values. Values of chroma (C*) and hue (H*) were also computed to determine the saturation and main hue of the fillet, respectively, according to the following formulae:

$$C^* = \sqrt{(a^{*2} + b^{*2})}$$

$$H^* = \arctan (b^*/a^*)$$

In addition, the colour of the trout fillets was assessed by comparison using the DSM SalmoFan (DSM Nutritional Products, Parsippany, NJ, USA), which adopts a scale ranging from 21 (light red) to 34 (dark red), under standardised conditions, employing a light cabinet fitted with a cool, daylight fluorescent light source. Three measurements of the colour of each fillet were carried out in the same position as those measured by means of the Minolta Chroma Meter instrument. This method was used because it is the easiest to apply and the best known and used by farmers. The left fillets were immediately stored in black plastic bags at -30°C for subsequent analysis. The carotenoid concentration and astaxanthin content in the diets and salmon fillets were determined chromatographically. Skinned fillets were ground with a meat mincer and frozen. Two samples of each fillet were accurately weighed (10±0.2 g) and repeatedly extracted with acetone (40 mL) until they were colourless. The pooled extracts were filtered and an aliquot (10 mL) was centrifuged at 1800 g for 5 min). The astaxanthin content in the supernatant was determined by means of a Varian ProStar instrument, equipped with a UV/vis detector, using an external astaxanthin standard at the detection wavelength of 470 nm. Analysis was performed on a Varian Kromasil 100 C18 250×0.3 mm column, according to Bjerkeng *et al.* (1997).

Proximate composition, cholesterol and fatty acid profile of the fillet

At the end of the trial a portion of about 50 g of skinless, left dorsal muscle was collected from each fish (10 fish per group), homogenised and submitted to proximate analysis (moisture, protein, lipid and ash content). The percentage of moisture was determined in duplicate according to the Association of Official Analytical Chemists procedure (AOAC, 1990). Proteins were determined using the standard Kjeldahl copper catalyst method (AOAC, 1990). Ashes were determined using the procedure described by the AOAC (AOAC, 1990). Total lipids were measured using a modification of the chloroform:methanol procedure described by Folch *et al.* (1956). After the determination of total lipids, fatty acids were converted to methyl esters following the method described by Christopherson and Glass (1969). The separation of fatty acids was carried out by using a Carlo Erba HRGC 5160 gas chromatograph (Carlo Erba Strumentazione, Rodano, MI, Italy) with a WP-4 Shimadzu integration system (Shimadzu Corporation, Tokyo, Japan), equipped with a Supelco SPTM - 2340 capillary column (30 m×0.32 mm i.d.; 0.20 µm film thickness; Supelco, Bellefonte, PA, USA) and a flame

ionisation detector. The operating conditions of the gas chromatograph were as follows: oven temperature was kept at 170°C for 15 min, increased to 190°C at a rate of 1°C/min, then increased to 220°C at a rate of 5°C/min and kept at this temperature for 17 min. The temperature of the injector was 270°C and the temperature of the detector was 300°C. Helium was used as the carrier gas at a constant flow of 1.7 mL/min. The identification of individual fatty acids was accomplished by a comparison of retention times to fatty methyl esters of standard mixtures (37 FAME Mix and C22:5 n3, Supelco). Cholesterol determination was carried out on the total lipid extract, following the method described by Manzi *et al.* (1996), by using a Shimadzu HPLC with a Shimadzu SPD-M10A Diode Array Detector equipped with an Alltech

Table 1. Chemical composition, pigment concentration and categories of fatty acids evaluated in the krill meal.

Moisture, %	10
Protein, % wet weight	57.8
Lipids, % wet weight	18.1
Ash, % wet weight	13.2
Astaxanthin, mg/kg	150
Saturated fatty acids, % of total fatty acids	34.9
Monounsaturated fatty acids, % of total fatty acids	27.1
Polyunsaturated fatty acids, % of total fatty acids	38
n-3 polyunsaturated fatty acids, % of total fatty acids	36.4

Table 2. Ingredients, proximate composition of the finisher diets administered to rainbow trout in the last 60 days of the rearing cycle.

	AS	KR
Ingredients		
Krill meal, %	-	60
Soybean extracted meal, %	40	-
Fish meal, %	34	5
Corn gluten, %	-	10
Wheat meal, %	14	8
Gelatinised wheat starch, %	6	5
Fish oil, %	2.5	5
Soybean oil, %	2.5	-
Whey powder, %	-	2.5
Wheat, %	-	3.5
Mineral vitamin premix, %	1	1
Proximate composition		
Moisture, %	8.0±0.2	7.0±0.2
Protein, % wet weight	44.1±0.9	44.3±0.6
Lipids, % wet weight	19.9±1.2	18.3±0.9
Ash, % wet weight	10±0.5	9.6±0.3
Pigment concentration, mg/kg feed	90	90

AS, astaxanthin diet; KR, krill diet.

Nucleosil C18 column (150 mm length; 4.6 mm i.d.; 5 µm thickness; Alltech Italia Srl, Sedriano, MI, Italy). The mobile phase was methanol: water (97:3, vol:vol) at a flow rate of 1.5 mL/min. The quantification of total cholesterol content was obtained by using an external calibration curve of β-sitosterol (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

The effect of the diet on the colour coordinates (L*, a*, b*, C*, H*), carotenoid and astaxanthin concentration, proximate composition and fatty acid profile was analysed by one-way analysis of variance (ANOVA) using the General Model procedure of SAS (SAS, 1988). Differences were considered significant at P<0.01 and means were separated by the Student Newmann-Keuls test.

Results

The physicochemical parameters of the water controlled in the two raceways always stayed within the range considered optimal for salmonids: water temperature was 14±2°C; dissolved oxygen was maintained above 8 ppm; pH ranged around 7.98±0.5; nitrogen compounds stayed below 0.40 mg/L (N-NH₃), 0.001 mg/L (N-NO₂) and 0.5 mg/L (N-NO₃); phosphates were around 0.08 ppm throughout the trial.

Diet composition

The proximate analysis of the two feeds is shown in Table 2: the compositions of the diets were very similar and they were in agreement with the requirements estimated by the National Research Council (Bromage and Shepherd, 1988). The fatty acid profile showed relevant variations particularly for the categories of saturated and polyunsaturated fatty acids (Table 3). The KR diet had a higher proportion of saturated fatty acids (+50%) than the AS diet owing to the higher proportions of myristic (C14:0) and palmitic acids (C16:0) (11.7 and 20.3% *vs* 4.63 and 14.70%, respectively). The total n-3 PUFA proportion was much higher in the KR diet owing to the higher proportions of both EPA and DHA, and consequently the n-3/n-6 PUFA ratio was more than four times higher in the KR diet compared to the AS diet (1.95 *vs* 0.41). The total PUFA proportion and n-6 PUFA noticeably increased in the AS diet because of the very high content of linoleic acid (C18:2 n-6): 27.33% *vs* 4.67%. This may be attributed to the use, in the feed formulation of the AS diet, of soybean meal and oil, notoriously rich in linoleic acid and lack-

ing in n-3 PUFA, in the replacement of fish meal and fish oil.

Colour measurements, carotenoid and astaxanthin concentration

The AS diet produced the highest accumulation of total carotenoids in the fillet compared

to the KR diet; the differences between the two groups were significant after 15 days of feeding (2.5 *vs* 2.1 mg/kg) till the end of the trial (5.8 *vs* 4.8 mg/kg). The same pattern was observed for the astaxanthin concentration with the highest values in the fillet of fish fed the AS diet (Table 4).

The colour coordinates of fillet are reported

Table 3. Fatty acid profile of the finisher feeds administered to rainbow trout in the last 60 days of the rearing cycle.

Diets	AS	KR
n	3	3
Fatty acid profile, % of total fatty acids		
Saturated		
C 14:0	4.60±0.02	11.70±3.60
C 15:0	0.30±0.03	0.50±0.04
C 16:0	14.70±1.10	20.30±1.80
C 17:0	0.50±0.01	1.80±0.04
C 18:0	4.00±0.03	2.10±0.06
C 20:0	0.40±0.02	0.20±0.04
C 21:0	0.10±0.03	0.10±0.03
C24:0	0.10±0.01	0.00±0.00
Total saturated	24.80±1.30	36.70±1.60
Monounsaturated		
C 14:1	0.20±0.03	0.30±0.07
C 15:1	0.05±0.01	0.10±0.02
C 16:1	5.10±0.06	8.10±0.04
C 17:1	0.80±0.02	1.00±0.02
C 18:1	19.40±0.12	19.80±0.40
C 20:1	1.10±0.02	2.10±0.03
C 24:1	0.40±0.02	0.40±0.01
Total monounsaturated	27.10±2.02	31.80±1.90
Polyenoic (n-3)		
C 18:3 n-3	1.02±0.01	1.83±0.04
C 20:5 n-3, EPA	6.83±0.57	9.02±1.60
C 22:5 n-3, DPA	1.32±0.04	0.60±0.02
C 22:6 n-3, DHA	4.01±0.72	6.56±1.30
Total n-3	13.17±1.31	18.01±2.80
Polyenoic (n-6)		
C 18:2 n-6	27.33±0.06	4.67±0.03
C 18:3 n-6	3.80±0.02	1.01±0.01
C 20:2 n-6	0.10±0.02	0.04±0.01
C 20:3 n-6	0.66±0.03	2.01±0.01
C 20:4 n-6	0.12±0.01	1.51±0.02
Total n-6	32.00±0.09	9.23±0.02
Total polyunsaturated	45.17±1.40	27.24±2.70
n-3/n-6	0.41±0.04	1.95±0.02
Others	2.90±0.03	4.17±0.02

AS, astaxanthin diet; KR, krill diet.

Table 4. Content of total carotenoids (mg/kg) and astaxanthin (mg/kg) in the fillet of rainbow trout fed the two finisher diets at different sampling times.

fish/sampling, n	Total carotenoids		Astaxanthin	
	AS	KR	AS	KR
Day 0	1.00±0.03	1.00±0.03	0.80±0.02	0.80±0.02
Day 15	2.50±0.01 ^A	2.10±0.02 ^B	2.30±0.06 ^A	1.90±0.02 ^B
Day 30	3.70±0.02 ^A	2.30±0.06 ^B	3.60±0.13 ^A	2.10±0.07 ^B
Day 45	4.70±0.01 ^A	3.50±0.04 ^B	4.20±0.06 ^A	3.30±0.05 ^B
Day 60	5.80±0.03 ^A	4.80±0.02 ^B	5.40±0.01 ^A	4.30±0.03 ^B

AS, astaxanthin diet; KR, krill diet; ^{A,B} different letters on the same line show significant differences (P<0.01).

in Table 5. Fillet lightness (L^*) was not affected by the trout diets whereas redness (a^*) and yellowness (b^*) were significantly higher in fish fed the AS diet until day 30 of the trial. Hue was not affected by the feeding treatment, whereas chroma was significantly higher in AS-fed fish throughout the trial with the exception of day 45 sampling. From the evaluation of fillet colour using the SalmoFan scale, it emerged that trout fed the diet containing krill meal had a paler pink-red colour than those receiving the diet containing synthetic astaxanthin.

Proximate composition, cholesterol and fatty acid profile of the fillet

No significant differences emerged regarding proximate composition and cholesterol content of the trout in the two groups (Table 6). The profile of the most important fatty acids of the rainbow trout, evaluated after 60 days of feeding with the two diets, is shown in Table 7. Total saturated fatty acids, primarily represented by palmitic acid (16:0), were significantly higher in the fillets of the KR-fed fish than in the AS-fed ones. The proportion of total monounsaturated fatty acids was similar in the fillets of the two groups. The percentage of n-6 PUFA in the AS-fed trout was twice that of the KR-fed trout (19.8 *vs* 9.8) owing to the higher proportion of linoleic acid (C18:2) (14.6% *vs* 6.7%). On the contrary, the proportions of EPA, DPA, DHA and total n-3 PUFA were significantly higher in KR-fed fish. The ratio n-3/n-6 PUFA was also markedly higher in trout fed the KR diet than fish fed the AS diet. In general, the fatty acid profile of the fillets reflected the fatty acid profile of the diets administered to the trouts.

Discussion

In our study, the use of krill meal in the feed for pigmentation of the fillet of the rainbow trout was investigated in the last 60 days of the rearing cycle. Trout receiving feed with krill meal had a paler pink-red colour than those receiving commercial astaxanthin. This difference was previously reported by Storebakken (1988), who explained this discrepancy as the form of krill astaxanthin, which is mostly in the esters of fatty acids, whereas salmonids utilise esterified astaxanthin to a lesser degree than free astaxanthin. On the contrary, Suontama *et al.* (2007) obtained fish with a deep red flesh colouration when the fish-meal protein of the control diet was replaced with 20% of the northern krill species (*Thysanoessa*

Table 5. Evaluation of fillet colour of rainbow trout fed on the two feeds through the CIE system and SalmoFan at the different sampling times.

	L^*	a^*	b^*	Hue	Chroma	SalmoFan
Day 0	41.6±2.3	0.6±1.1	3.0±1.5	0.3±0.03	4.1±1.16	19.5±0.5
Day 15						
AS	42.3±1.6	3.2±1.6 ^A	2.6±0.2 ^A	0.8±0.3	4.3±1.3 ^A	22.8±0.06 ^A
KR	42.1±1.2	1.0±0.6 ^B	1.1±1 ^B	0.9±0.3	1.2±0.09 ^B	20.1±0.05 ^B
Day 30						
AS	41.2±1.5	4.1±0.4 ^A	4.9±0.8 ^A	0.9±0.1	6.4±0.4 ^A	24.7±0.04 ^A
KR	42.1±3.5	2.9±0.7 ^B	2.0±0.6 ^B	0.6±0.2	3.6±0.6 ^B	22.6±0.08 ^B
Day 45						
AS	41.6±1.2	5.8±1.7	5.5±2.1	0.8±0.3	8.2±1.6	28.6±0.06 ^A
KR	42.5±2.7	4.9±1.6	5.0±1.3	0.8±0.1	6.9±1.3	26.4±0.07 ^B
Day 60						
AS	42.4±1.9	6.7±1.5	6.2±2	0.7±0.1	10.5±1.3 ^A	30.8±0.03 ^A
KR	43.6±2.8	5.7±1.4	5.9±1.6	0.8±0.2	7.6±1.3 ^B	27.7±0.09 ^B

CIE system, L^* = lightness, a^* = redness and b^* = yellowness; ^{A,B} different letters on the same line show significant differences ($P < 0.01$).

inermis). In our experiment, following the application of the L^* , a^* , b^* system, the L^* parameter appeared the least suitable among the chromatic indices to highlight differences in the intensification of colour in the muscle of the rainbow trout. In this salmonid species, colourimetric measurements showed large variations in fillet colour, irrespective of dietary treatment: L^* values ranged from 35 to 63, a^* from 5 to 26 and b^* from 7 to 31. Large variations in colour measures were found by Schubring (2009) who found that the L^* measure is significantly correlated with flesh lipid content. On the contrary, according to Christiansen *et al.* (1995), the high fat content in farmed salmon causes dilution of astaxanthin and interferes with colour perception. In our experiment, we did not find any correlation between colour measurements and fat content of the flesh. Other papers reported that muscle colour attributes were more affected by the astaxanthin source than by the oil source; this was reported by Choubert *et al.* (2006) who ascertained that trout fed on synthetic astaxanthine showed higher chroma (C^*), redness and yellowness than fish fed on feed supplemented with astaxanthin from the green micro-algae *Haematococcus pluvialis*. Using other natural pigmentation compounds, such as red pepper, in comparison with commercial astaxanthin, Yanar *et al.* (2007) observed that the fillet colour was lighter. Similar results were obtained by Akhtar *et al.* (1999), who observed that paprika, compared with canthaxanthine, produced less pigmentation of the muscle with higher values in lightness and redness.

In our trial, the fillet colour measured by means of SalmoFan showed significantly higher scores in fish receiving the synthetic asta-

Table 6. Proximate composition and total cholesterol amount in fillets of rainbow trout fed the two finisher feeds.

	AS	KR
Moisture, g/100 g	76.6±0.5	76.3±0.3
Protein, g/100 g	19.4±0.6	19.5±0.2
Lipids, g/100 g	3.3±0.5	3.5±0.4
Ash, g/100 g	1.3±0.2	1.3±0.1
Cholesterol, mg/100 g	57.0±0.8	56.7±5.8

AS, astaxanthin diet; KR, krill diet.

xanthine. In Atlantic salmon, SalmoFan was able to score an increase in colour when increasing proportions of canthaxanthin were added to the feed compared with astaxanthin (Buttle *et al.*, 2001). In the same species, some interesting studies showed a significant linear correlation between the SalmoFan score and muscle astaxanthin concentration (Christiansen *et al.*, 1995). A study carried out by Forsberg and Guttormsen (2006) modelled the relationships between muscle astaxanthin concentration and visual colour perception (SalmoFan score), using the explanatory variables dietary astaxanthin concentration and fish size, and showed that the two dependent variables gave an excellent fit to the observed data. In another experiment (Misimi *et al.*, 2007), the comparisons between fillet colour evaluated through visual measures by a panel of human inspectors, according to the SalmoFan standard, and the colour scores generated from a computer vision algorithm confirmed that there were no significant differences between the two methods. However, the SalmoFan method proved unsuitable when farmed and wild salmon were compared, as observed by Johnston *et al.* (2006) who did not

find differences between farmed and wild salmon: flesh colour measured with SalmoFan ranged between 27.4 and 28.0, even if total carotenoid pigment concentrations were considerably higher in farmed (7.68-8.43 mg/kg) than in wild (5.53-6.44 mg/kg) salmon.

In relation to carotenoid pigments and astaxanthin content, although in our experiment fish receiving natural pigments exhibited a paler muscle colour than those fed a diet containing synthetic astaxanthin, the amounts of carotenoids and astaxanthin contained in the diet were considered to be adequate for the pigmentation of the rainbow trout. In other studies (Yanar *et al.*, 2007), carried out using natural pigment compounds such as red pepper and marigold flower, the carotenoid contents found in the fish fillet ranged from 1.5 to 5.6 mg/kg depending on the application time, but synthetic astaxanthin provided the highest carotenoid accumulation in the fish followed by red pepper. In the rainbow trout, canthaxanthin was more efficiently absorbed (3.8-7.9 mg/kg) in the flesh than the paprika carotenoids (2.4-3.1 mg/kg) (Akhtar *et al.*, 1999). In Atlantic salmon, the final total carotenoid concentration of the muscle was significantly higher in fish fed the diet supplemented with *P. rhodozyma* (2.56 mg/kg) compared to salmon fed a diet supplemented with a source of chemically synthesised astaxanthin (1.96 mg/kg) (Bjerkeng *et al.*, 2007).

As regards the quality traits of the fillets, we found a very similar proximate composition between trout receiving the experimental and commercial feeds, showing it to be affected by the dietary composition as documented in the literature (Rasmussen, 2001). The protein content was nearly equal to that reported for rainbow trout at market size, whereas the lipid content appeared lower than data reported both by Yanar *et al.* (2007) and by Chaiyapechara *et al.* (2003); the latter ascertained a proportion of 8.8% of lipids in the trout fed on a commercial diet based on a 17% lipid content.

Krill meal provided a high quality product from the nutritional point of view, because it improved the fatty acid profile. The trout fed the diet based on krill meal showed a notable content of essential n-3 PUFA. The fatty acid profile showed a very high proportion of EPA and DHA, and a low rate of linoleic acid with a favourable n-3/n-6 PUFA ratio. These results appeared more satisfactory than those reported by Yanar *et al.* (2007) who, using a basal diet containing 45% crude protein, 10% crude fat, supplemented with commercial astaxanthin and natural pigments, obtained EPA levels ranging from 5.73% (red

pepper) to 5.95% (commercial astaxanthin). Studies carried out in rainbow trout reared in freshwater and seawater cages (Haliloğlu *et al.*, 2004) also reported lower EPA contents and PUFA than those obtained in our experiment. This demonstrates that krill meal has a satisfactory nutritional value and also appears to be useful for inclusion in the diet for pigmenting rainbow trout.

Conclusions

In this paper, krill meal was used for pigmenting the trout fillet; even if the pigmentation was lighter pink, this colour is appreciated by the consumers of organic products who are adverse to the use of synthetic products in ani-

mal feeding. Moreover, krill meal enhanced the flesh amount of n-3 PUFA considered beneficial for human health. Krill meal could therefore represent an alternative to synthetic sources for flesh pigmentation in organic aquaculture, as contemplated in the recent EC Regulation No 710/2009 of 5 August 2009 (European Commission, 2009) that admits the use of astaxanthin derived primarily from organic sources, such as organic crustacean shells, in the feed ration for salmon and trout within the limit of their physiological needs.

This consideration is in agreement with the guidelines regarding organic production, as reported by a survey of the European Commission in 2000, as well as with the relationship between organic fish feeds and environmental sustainability, focused on during the International Federation of Organic

Table 7. Fatty acid profile of fillets of rainbow trout fed the two finisher feeds.

	AS	KR
Fatty acid profile, % of total fatty acids		
Saturated		
C 11:0	0.02±0.04	0.03±0.03
C 12:0	0.01±0.02	0.02±0.03
C 14:0	4.80±0.10	5.40±0.20
C 15:0	0.20±0.08	0.40±0.01
C 16:0	15.60±0.30 ^B	18.50±0.10 ^A
C 17:0	0.40±0.10	0.50±0.02
C 18:0	3.10±0.06	4.30±0.02
C 20:0	0.50±0.10	0.40±0.04
C 21:0	0.90±0.03 ^A	0.30±0.02 ^B
C 24:0	0.30±0.30	0.00±0.00
Total saturated	25.80±0.30 ^B	29.90±0.10 ^A
Monounsaturated		
C 14:1	0.10±0.01	0.10±0.10
C 15:1	0.00±0.00 ^B	0.09±0.01 ^A
C 16:1	3.60±0.60 ^B	6.70±0.10 ^A
C 17:1	0.50±0.10 ^B	0.90±0.05 ^A
C 18:1	23.60±1.50 ^A	19.30±0.20 ^B
C 20:1	1.70±0.70	1.30±0.02
C 24:1	0.20±0.10 ^B	0.60±0.02 ^A
Total monounsaturated	29.70±2.40	29.10±0.30
Polyenoic (n-3)		
C 18:3 n-3	2.70±0.40 ^A	0.50±0.06 ^B
C 20:5 n-3, EPA	4.80±0.30 ^B	8.90±0.70 ^A
C 22:5 n-3, DPA	1.50±0.20 ^B	3.50±0.40 ^A
C 22:6 n-3, DHA	13.50±0.80 ^B	16.40±1.00 ^A
Total n-3	22.40±1.50 ^B	29.40±1.02 ^A
Polyenoic (n-6)		
C 18:2 n-6	14.60±0.50 ^A	6.70±0.09 ^B
C 18:3 n-6	2.50±0.50 ^A	1.00±0.06 ^B
C 20:2 n-6	0.70±0.00 ^A	0.20±0.02 ^B
C 20:3 n-6	1.30±0.20 ^A	0.90±0.02 ^B
C 20:4 n-6	0.70±0.20 ^B	1.00±0.03 ^A
Total n-6	19.80±0.50 ^A	9.80±0.10 ^B
Total polyunsaturated	42.20±1.80 ^A	39.20±0.10 ^B
n-3/n-6 ratio	1.10±0.07 ^B	3.00±0.10 ^A

AS, astaxanthin diet; KR, krill diet; ^{A,B} different letters on the same line show significant differences (P<0.01).

Agriculture Movements (IFOAM) World Congress (Bridson, 2008). Moreover, considering that the krill biomass worldwide production is estimated to be as high as 500 million tons, and still represents an unexploited source (only about 12% of it is consumed) (Ichii, 2000), it appears to be a valuable ingredient in organic fish feeding and particularly in trout production.

References

- Akhtar, P., Gray, J.I., Cooper, T.H., Garling, D.L., Booren, A.M., 1999. Dietary pigmentation and deposition of α -tocopherol and carotenoids in rainbow trout muscle and liver tissue. *J. Food Sci.* 64:234-239.
- American Water Works Association and Water Pollution Control Federation of American Public Health Association, 1989. Standard methods for the examination of water and wastewater. 17th ed. APHA Publ., Washington, DC, USA.
- Anderson, S., 2000. Salmon color and the consumer. pp 1-3 in Proc. 10th IIFET Conf., Oregon State University Publ., Corvallis, OR, USA.
- Anderson, J.S., Richardson, N.L., Higgs, D.A., Dosanjh, B.S., 1997. The evaluation of air-dried krill meal as a dietary protein supplement for juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Can. Tec. Rep. Fish. Aquat. Sci.* 17:17-33.
- AOAC, 1990. Meat and meat products. In: Official Methods of Analysis. Vol.2., 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- Bjerkeng, B., Peisker, M., von Schwartzberg, K., Ytrestøl, T., Åsgård, T., 2007. Digestibility and muscle retention of astaxanthin in Atlantic salmon, *Salmo salar*, fed diets with the red yeast *Phaffia rhodozyma* in comparison with synthetic formulated astaxanthin. *Aquaculture* 269:476-489.
- Bjerkeng, B., Refstie, S., Fjalestad, K.T., Storebakken, T., Rodbotten, M., Roem, A.J., 1997. Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. *Aquaculture* 157:297-309.
- Bridson, P., 2008. Organic aquaculture feeds - towards proven sustainability. pp 47-50 in Proc. 16th IFOAM Organic World Congr., Cattolica (RN), Italy.
- Bromage, N., Shepherd, J., 1988. Fish, their requirements and site evaluation. In: J. Shepherd and N. Bromage (eds.) *Intensive Fish Farming*. BSP Professional Books, Oxford, UK, pp 36-39.
- Buttle, L.G., Crampton, V.O., Williams, P.D., 2001. The effect of feed pigment type on flesh pigment deposition and colour in farmed Atlantic salmon, *Salmo salar* L. *Aquac. Res.* 32:103-111.
- Chaiyapechara, S., Casten, M.T., Hardy, R.W., Dong, F.M., 2003. Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentrations of lipid and vitamin E. *Aquaculture* 219:715-738.
- Choubert, G., Heinrich, O., 1993. Carotenoid pigments of the green alga *Haematococcus pluvialis*: assay on rainbow trout, *Oncorhynchus mykiss*, pigmentation in comparison with synthetic astaxanthin and canthaxanthin. *Aquaculture* 112:217-236.
- Choubert, G., Mendes-Pinto, M.M., Morais, R., 2006. Pigmenting efficacy of astaxanthin fed to rainbow trout *Oncorhynchus mykiss*: Effect of dietary astaxanthin and lipid sources. *Aquaculture* 257:429-436.
- Christiansen, R., Struknaes, G., Estermann, R., Torrisen, O.J., 1995. Assessment of flesh colour in Atlantic salmon *Salmo salar* L. *Aquaculture Research* 26:311-321.
- Christopherson, S.W., Glass, R.L., 1969. Preparation of milk methyl esters by alcoholysis in an essentially non-alcoholic solution. *J. Dairy Sci.* 52:1289-1290.
- Commission International de L'Eclairage, 1978. Recommendations on uniform colour spaces, colour difference equations, psychometric colour terms. CIE No. E-1.3.1, 1971/(TO-1.3). CIE Ed., Paris, France.
- Diler, I., Gökoğlu, N., 2004. Investigation of sensory properties of the flesh of rainbow trout (*Oncorhynchus mykiss*) fed diets with astaxanthin, shrimp waste meal and red pepper meal. *Eur. Food Res. Technol.* 219:217-222.
- European Commission, 2003. Commission Directive of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council of undesirable substances in animal feed. In: Official Journal, L285, 1/11/2003, pp 33-37.
- European Commission, 2009. Regulation No. 710/2009 of 5 August 2009 amending Regulation No. 889/2008, laying down detailed rules for the implementation of Council Regulation, EC No. 834/2007. In: Official Journal, L204, 6/8/2009, pp 15-34.
- European Food Safety Authority, 2007. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the Maximum Residue Limits for canthaxanthin in foodstuffs coming from animals fed with canthaxanthin used as a feed additive in accordance with Council Directive 70/524/EEC. In: *The EFSA Journal* 507, pp 1-19.
- Folch, J., Lees, M., Sloane Stanley, G.H., 1956. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 60:497-509.
- Forsberg, O.I., Guttormsen, A.G., 2006. A pigmentation model for farmed Atlantic salmon: nonlinear regression analysis of published experimental data. *Aquaculture* 253:415-420.
- Gentles, A., Haard, N., 1990. Bioavailability of astaxanthin in *Phaffia rhodozyma*. In: J.R. Botta and M.N. Voigt (eds.) *Advances in Fisheries Technology and Biotechnology for Increased Profitability*. Technomic Publ. Co. Inc., Lancaster, PA, USA, pp 373-386.
- Georgakopoulos, G., Thomson, I., 2005. Organic salmon farming: risk perceptions, decision heuristics and the absence of environmental accounting. *Accounting Forum* 29:49-75.
- Gigliotti, J.C., Jaczynski, J., Tou, J.C., 2008. Determination of the nutritional value, protein quality and safety of krill protein concentrate isolated using an isoelectric solubilization/precipitation technique. *Food Chem.* 111:209-214.
- Haliloğlu, H.I., Bayir, A., Sirkecioglu, A.N., Aras, N.M., Atamanalp, M., 2004. Comparison of fatty acid composition in some tissues of rainbow trout (*Oncorhynchus mykiss*) living in seawater and freshwater. *Food Chem.* 86:55-59.
- Ichii, T., 2000. Krill harvesting. In: I. Everson (ed.) *Krill: Biology, Ecology and Fisheries - Fish and Aquatic Resources*. Wiley Online Library DOI: 10.1002/9780470999493.ch9.
- Johnston, I.A., Li, X., Vieira, V.L.A., Nickell, D., Dingwall, A., Alderson, R., Campbell, P., Bickerdike, R., 2006. Muscle and flesh quality traits in wild and farmed Atlantic salmon. *Aquaculture* 256:323-336.
- Maki, K.C., Reeves, M.S., Farmer, M., Griinari, M., Berge, K., Vik, H., Hubacher, R., Rains, T.M., 2009. Krill oil supplementation increases plasma concentrations of eicosapentaenoic and docosahexaenoic acids in overweight and obese men and women. *Nutr. Res.* 29:609-615.
- Manzi, R., Panfili, G., Pizzoferrato, L., 1996. Normal and reversed-phase HPLC for more complete evaluation of tocopherols,

- retinols, carotenes and sterols in dairy products. *Chromatographia* 43:89-93.
- Misimi, E., Mathiassen, J.R., Erikson, U., 2007. Computer vision-based sorting of Atlantic salmon (*Salmo salar*) fillets according to their color level. *J. Food Sci.* 72:S30-S35.
- Moren, M., Suontama, J., Hemre, G.-I., Karlsen, Ø., Olsen, R.E., Mundheim, H., Julshamn, K., 2006. Element concentrations in meals from krill and amphipods - possible alternative protein sources in complete diets for farmed fish. *Aquaculture* 261:174-181.
- Nickell, D.C., Springate, J.R.C., 2001. Pigmentation of farmed salmonids. In: S. Kestin and P. Wariss (eds.) *Farmed Fish Quality*. Blackwell Science, Oxford, UK, pp 58-75.
- Rasmussen, R.S., 2001. Quality of farmed salmonids with emphasis on proximate composition, yield and sensory characteristics. *Aquac. Res.* 32:767-786.
- SAS, 1998. *Guide for Personal Computers, Version 6.03* SAS Inst. Inc., Cary, NC, USA.
- Schubring, R., 2009. Colour measurement. In: H. Rehbein and J. Oehlenschläger (eds.) *Fishery Products. Quality, Safety and Authenticity*. Wiley Blackwell, Oxford, UK, pp 127-172.
- Scott, T.M., Rasco, B.A., Hardy, R.W., 1994. Stability of krill meal, astaxanthin and canthaxanthin color in cultured rainbow trout (*Oncorhynchus mykiss*) fillets during frozen storage and cooking. *J. Aquat. Food Prod. T.* 3:53-63.
- Storebakken, T., 1988. Krill as a potential feed source for salmonids. *Aquaculture* 70:193-205.
- Storebakken, T., Foss, P., Schiedt, K., Austreng, E., Liaaen-Jensen, S., Manz, U., 1987. Carotenoids in diets for salmonids. IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. *Aquaculture* 65:279-292.
- Suontama, J., Kiessling, A., Melle, W., Waagbo, R., Olsen, R.E., 2007. Protein from Northern krill (*Thysanoessa inermis*), Antarctic krill (*Euphausia superba*) and the Arctic amphipod (*Themisto libellula*) can partially replace fish meal in diets to Atlantic salmon (*Salmo salar*) without affecting product quality. *Aquac. Nutr.* 13:50-58.
- Tibaldi, E., Ballestrazzi, R., 1990. Effects of the levels of astaxanthin and of lipids in the diet on the pigmentation of rainbow trout. *Zoot. Nutr. Anim.* 16:127-135.
- Torrissen, O.J., 1989. Pigmentation of salmonids - interactions of astaxanthin and canthaxanthin on pigment deposition in rainbow trout. *Aquaculture* 79:363-374.
- Torrissen, O.J., Christiansen, R., Struksnæs, G., Estermann, R., 1995. Astaxanthin deposition in the flesh of Atlantic salmon, *Salmo salar* L., in relation to dietary astaxanthin concentration and feeding period. *Aquac. Nutr.* 1:77-84.
- Yanar, Y., Büyükçapar, H., Yanar, M., Göcer, M., 2007. Effect of carotenoids from red pepper and marigold flower on pigmentation, sensory properties and fatty acid composition of rainbow trout. *Food Chem.* 100:326-330.
- Yoshitomi, B., Aoki, M., Oshima, S., 2007. Effect of total replacement of dietary fish meal by low fluoride krill (*Euphausia superba*) meal on growth performance of rainbow trout (*Oncorhynchus mykiss*) in fresh water. *Aquaculture* 266:219-225.

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