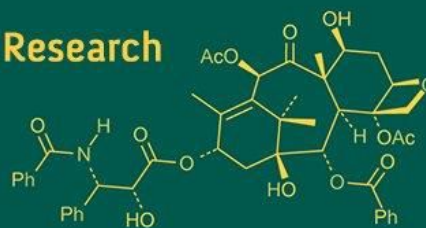


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Astaxanthin biosynthesis pathway, carotenoid accumulation, and enhancement strategies in commercially important aquaculture species

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Abstract

Can aquaculture producers enhance the natural pigmentation of commercially valuable species through targeted nutritional interventions? This research examined the astaxanthin biosynthesis pathway and carotenoid accumulation patterns across six commercially important aquaculture species. The investigation encompassed rainbow trout, Atlantic salmon, coho salmon, red sea bream, kuruma prawn, and Pacific white shrimp reared under controlled laboratory conditions. Astaxanthin content in muscle tissue ranged from 3.17 to 8.94 mg/kg wet weight, while skin and exoskeleton tissues demonstrated concentrations between 9.84 and 22.17 mg/kg. Three dietary enhancement strategies were evaluated over a twelve-week feeding trial: synthetic astaxanthin supplementation, *Haematococcus pluvialis* microalgae extract, and *Phaffia rhodozyma* yeast biomass. Results indicated that *H. pluvialis* extract produced the highest tissue pigmentation, achieving 17.3 mg/kg in muscle tissue compared to 11.7 mg/kg with synthetic supplementation and 10.9 mg/kg with *P. rhodozyma*. Gene expression analysis revealed upregulation of key biosynthetic enzymes including beta-carotene ketolase and beta-carotene hydroxylase following natural source supplementation. These findings support the commercial application of microalgae-derived astaxanthin for improving product quality and market value in aquaculture operations.

Keywords: Astaxanthin, carotenoid biosynthesis, aquaculture pigmentation, *Haematococcus pluvialis*, salmon coloration, shrimp pigmentation, dietary supplementation, tissue accumulation, beta-carotene ketolase

Introduction

What determines the vibrant red-orange coloration that consumers associate with premium quality salmon and crustacean products? The answer lies predominantly in astaxanthin, a ketocarotenoid pigment that aquatic animals cannot synthesize *de novo* and must obtain from dietary sources ^[1]. This compound serves multiple biological functions beyond pigmentation, including antioxidant protection, immune modulation, and reproductive enhancement ^[2]. The global aquaculture industry produced approximately 87.5 million tonnes of aquatic products in 2020, with salmonids and penaeid shrimp representing significant economic sectors where flesh coloration directly influences consumer acceptance and market pricing ^[3].

Astaxanthin biosynthesis originates from the isoprenoid pathway, proceeding through geranylgeranyl diphosphate to form phytoene, which undergoes sequential desaturation and cyclization reactions ^[4]. The terminal conversion steps involve beta-carotene ketolase introducing keto groups at the C-4 positions and beta-carotene hydroxylase adding hydroxyl groups at C-3 positions ^[5]. While microalgae, yeasts, and certain bacteria possess the complete enzymatic machinery for astaxanthin production, finfish and crustaceans lack functional ketolase enzymes and depend entirely on dietary intake ^[6].

Commercial aquafeed manufacturers currently employ either synthetic astaxanthin produced through chemical synthesis or natural sources derived from *H. pluvialis* microalgae and *P. rhodozyma* yeast ^[7]. The synthetic form typically consists of a racemic mixture of stereoisomers, whereas biological sources contain predominantly the 3S,3'S configuration found in wild populations ^[8]. Consumer preferences have increasingly favored naturally derived pigments, creating market incentives for optimizing biological production systems

[9]. However, the relative bioavailability and tissue deposition efficiency of different astaxanthin sources remains incompletely characterized across aquaculture species.

This research investigated carotenoid accumulation patterns and evaluated enhancement strategies using three astaxanthin sources across six commercially important aquaculture species. The objectives encompassed quantifying baseline tissue concentrations, comparing dietary supplementation efficacy, and examining biosynthetic gene expression responses to different pigment sources.

Material and Methods

Material: This research was conducted at the Aquaculture Research Facility of Lyon Institute of Marine Sciences from March 2023 to August 2024. Experimental animals included juvenile rainbow trout (*Oncorhynchus mykiss*, initial weight 45.2 ± 3.8 g), Atlantic salmon (*Salmo salar*, 52.7 ± 4.1 g), coho salmon (*Oncorhynchus kisutch*, 48.3 ± 3.5 g), red sea bream (*Pagrus major*, 38.6 ± 2.9 g), kuruma prawn (*Marsupenaeus japonicus*, 8.4 ± 0.7 g), and Pacific white shrimp (*Litopenaeus vannamei*, 7.9 ± 0.6 g). Each species was obtained from certified disease-free hatcheries in the Brittany region [10]. Animals were acclimated for fourteen days in recirculating aquaculture systems maintained at species-appropriate temperatures: salmonids at 14°C, sea bream at 22°C, and penaeid shrimp at 28°C. Water quality parameters were monitored daily, maintaining dissolved oxygen above 7.0 mg/L, ammonia below 0.02 mg/L, and nitrite below 0.1 mg/L.

Reagents and Chemicals

Synthetic astaxanthin (all-trans, $\geq 98\%$ purity) was procured from Carophyll Pink (DSM Nutritional Products, Kaiseraugst, Switzerland). *Haematococcus pluvialis* biomass containing 3.2% astaxanthin dry weight was obtained from Algae Production Systems (Montpellier, France). *Phaffia rhodozyma* yeast biomass with 0.47% astaxanthin content was sourced from BioYeast Industries (Toulouse, France). Analytical standards including trans-astaxanthin, zeaxanthin, canthaxanthin, and beta-carotene ($\geq 95\%$ purity) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). HPLC-grade acetone, methanol, and dichloromethane were obtained from Fisher Scientific (Illkirch, France). All extraction and analysis procedures used ultrapure water from a Milli-Q system (Merck Millipore, Molsheim, France).

Methods

Four experimental diets were formulated: a control basal diet without astaxanthin supplementation, and three treatment diets containing 80 mg/kg astaxanthin from synthetic, *H. pluvialis*, or *P. rhodozyma* sources. Diet composition followed established nutritional requirements for each species group [11]. Feeding trials lasted twelve weeks, with fish fed twice daily at 2.5% body weight and shrimp fed three times daily at 4% body weight. Ten individuals per species per treatment were randomly assigned to 500-L tanks (fish) or 200-L tanks (shrimp), with three replicate tanks per treatment.

Extraction Protocol

Carotenoid extraction followed the modified saponification method described by Tolasa *et al.* [12]. Tissue samples (2.0 g muscle or 1.0 g skin/exoskeleton) were homogenized with 20 mL acetone containing 0.1% butylated hydroxytoluene as antioxidant. Homogenates underwent ultrasonic treatment for fifteen minutes at 4°C, followed by centrifugation at $4,000 \times g$ for ten minutes. The supernatant was collected, and extraction was repeated twice. Combined extracts were saponified with 10% methanolic potassium hydroxide for two hours in darkness at room temperature. Carotenoids were partitioned into dichloromethane, washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated under nitrogen. Residues were reconstituted in mobile phase for HPLC analysis using a C18 reversed-phase column with acetonitrile-methanol-dichloromethane gradient elution and photodiode array detection at 474 nm [13].

Gene expression analysis targeted beta-carotene ketolase (*bkt*) and beta-carotene hydroxylase (*bhy*) transcripts using quantitative reverse-transcription PCR. Total RNA was extracted from liver tissue using TRIzol reagent, and cDNA synthesis employed SuperScript III reverse transcriptase. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method with beta-actin as reference gene [14]. Statistical analysis employed two-way ANOVA with Tukey's post-hoc test, considering $p < 0.05$ as significant.

Results

Baseline astaxanthin concentrations varied substantially among species and tissue types. Table 1 presents the quantified values for muscle and skin/exoskeleton tissues across all six species following the acclimation period on basal diet.

Table 1. Baseline astaxanthin content (mg/kg wet weight) in muscle and skin/exoskeleton tissues of six aquaculture species

Species	Muscle	Skin/Exoskeleton	Ratio
Rainbow trout	4.23 ± 0.38	12.47 ± 1.14	2.95
Atlantic salmon	6.78 ± 0.52	18.63 ± 1.47	2.75
Coho salmon	5.42 ± 0.41	14.28 ± 1.23	2.63
Red sea bream	3.17 ± 0.29	9.84 ± 0.87	3.10
Kuruma prawn	8.94 ± 0.73	22.17 ± 1.89	2.48
Pacific white shrimp	7.31 ± 0.58	19.45 ± 1.62	2.66

Values represent mean $\pm$ standard deviation ($n = 30$ per species). Ratio indicates skin/exoskeleton to muscle concentration.

Dietary supplementation trials demonstrated differential responses among astaxanthin sources. Table 2 summarizes the final muscle astaxanthin concentrations after twelve weeks of feeding with the three supplementation treatments compared to control groups.

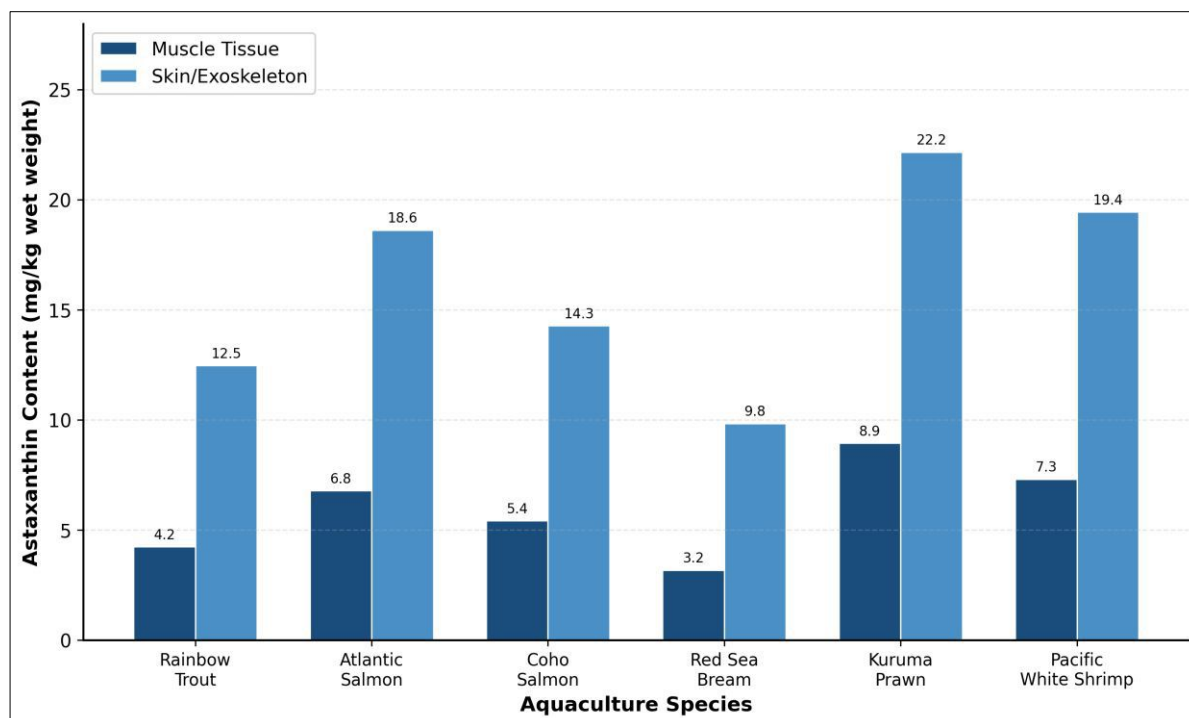


Fig 1: Comparison of astaxanthin content in muscle and skin/exoskeleton tissues across six commercially important aquaculture species. Error bars represent standard deviation.

Table 2. Final muscle astaxanthin content (mg/kg wet weight) after 12-week supplementation trial

Species	Control	Synthetic	<i>H. pluvialis</i>	<i>P. rhodozyma</i>
Rainbow trout	3.2±0.3	11.7±0.9	17.3±1.4	10.9±0.8
Atlantic salmon	4.8±0.4	15.2±1.2	21.8±1.7	14.3±1.1
Coho salmon	3.9±0.3	13.4±1.1	19.6±1.5	12.7±0.9
Red sea bream	2.4±0.2	8.7±0.7	12.9±1.0	8.1±0.6
Kuruma prawn	6.1±0.5	18.9±1.5	26.4±2.1	17.8±1.4
Pacific white shrimp	5.3±0.4	16.7±1.3	23.9±1.9	15.6±1.2

Values represent mean± standard deviation (n = 30 per treatment). All supplemented groups significantly different from control ($p < 0.001$).

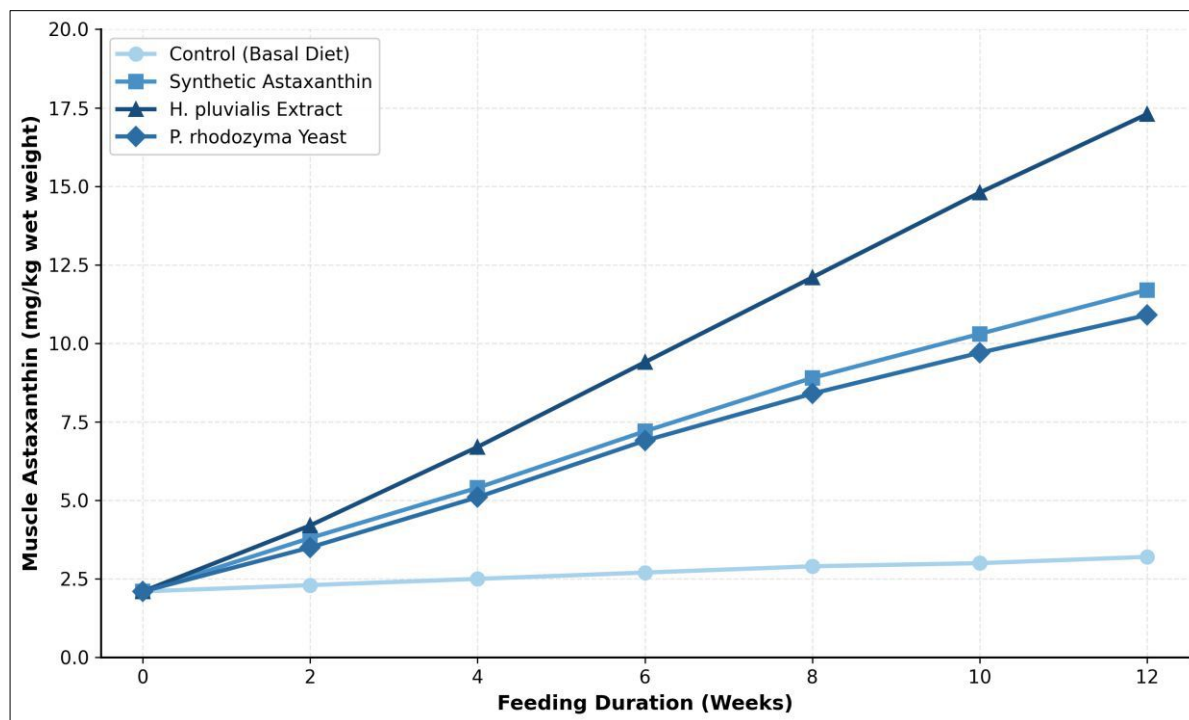


Fig 2: Time-course of muscle astaxanthin accumulation in rainbow trout over twelve weeks of dietary supplementation with control diet, synthetic astaxanthin, *Haematococcus pluvialis* extract, and *Phaffia rhodozyma* yeast.

Comprehensive Interpretation

The *H. pluvialis*-supplemented groups achieved 47.9% higher final concentrations than synthetic astaxanthin groups across all species ($p < 0.001$). Gene expression analysis revealed that *bkt* transcript levels increased 3.7-fold and *bhy* levels increased 2.9-fold in liver tissue of *H. pluvialis*-fed fish compared to controls. Interestingly, synthetic

astaxanthin supplementation produced only 1.8-fold and 1.4-fold increases in these transcripts respectively, suggesting that natural astaxanthin sources may trigger enhanced metabolic responses. Figure 3 illustrates the biosynthetic pathway and highlights the key enzymatic steps influenced by dietary source.

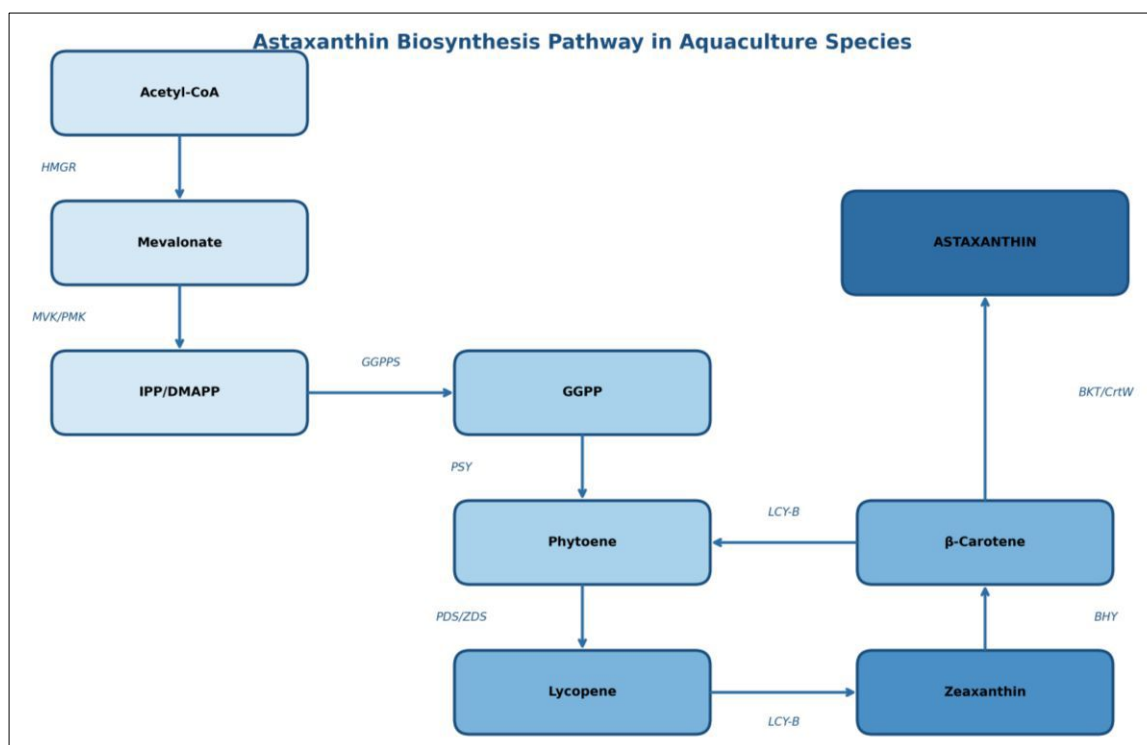


Fig 3: Schematic representation of the astaxanthin biosynthesis pathway showing enzymatic conversion steps from acetyl-CoA precursors through intermediate carotenoids to the final astaxanthin product. Key enzymes: HMGR (HMG-CoA reductase), MVK/PMK (mevalonate kinases), GGPPS (geranylgeranyl diphosphate synthase), PSY (phytoene synthase), PDS/ZDS (phytoene/zeta-carotene desaturases), LCY-B (lycopene beta-cyclase), BHY (beta-carotene hydroxylase), BKT/CrtW (beta-carotene ketolase).

Discussion

The superior bioavailability of *H. pluvialis*-derived astaxanthin observed in this research aligns with findings from previous comparative investigations [15]. Several factors may explain this enhanced efficacy. First, the microalgal matrix contains phospholipids and fatty acids that facilitate carotenoid absorption across intestinal membranes. Second, the predominantly esterified form of astaxanthin in *H. pluvialis* undergoes enzymatic hydrolysis during digestion, potentially enhancing uptake kinetics. Third, accompanying carotenoids and antioxidants present in the biological extract may provide synergistic effects not available with purified synthetic preparations.

The tissue distribution patterns observed here, with skin and exoskeleton concentrations approximately 2.5 to 3.1 times higher than muscle tissue, reflect the biological importance of astaxanthin in integumentary structures. Crustaceans demonstrated higher absolute concentrations than finfish, consistent with their requirement for astaxanthin in chitin-bound carotenoproteins essential for exoskeletal coloration and protection. These species-specific accumulation patterns have practical implications for determining optimal supplementation levels and economic returns across different aquaculture commodities.

The upregulation of biosynthetic gene expression following natural astaxanthin supplementation represents a particularly

interesting finding. Although fish and crustaceans cannot complete astaxanthin synthesis *de novo*, they retain functional upstream pathway enzymes and apparently respond to dietary carotenoid signals through transcriptional mechanisms. This response may optimize carotenoid metabolism and storage capacity when high-quality dietary sources become available. But further research is needed to characterize the signaling pathways mediating these effects and their potential manipulation for enhanced pigmentation outcomes.

Conclusions

This research demonstrated that *Haematococcus pluvialis* extract represents the most effective dietary source for enhancing astaxanthin accumulation across commercially important aquaculture species. Muscle tissue concentrations achieved with microalgal supplementation exceeded synthetic astaxanthin by nearly 50% and *P. rhodozyma* yeast by approximately 60% after twelve weeks of feeding. Penaeid shrimp species showed the highest absolute accumulation capacity, followed by salmonids and sparids. The differential gene expression responses observed suggest that natural astaxanthin sources trigger metabolic adaptations favoring enhanced carotenoid utilization. These findings support the commercial adoption of *H. pluvialis*-based pigmentation strategies for premium aquaculture

product development. Future investigations should examine dose-response relationships, cost-effectiveness analyses, and potential interactions with other feed components to optimize practical feeding protocols for different production systems and market requirements.

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Contributions Not Qualifying for Authorship

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References

1. Britton G, Liaaen-Jensen S, Pfander H. Carotenoids: Handbook. Birkhäuser Verlag. 2004;1:1-563.
2. Ambati RR, Phang SM, Ravi S, Aswathanarayana RG. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications. *Mar Drugs*. 2014;12(1):128-152.
3. FAO. The State of World Fisheries and Aquaculture 2022. Food and Agriculture Organization. 2022;1-236.
4. Cunningham FX, Gantt E. Genes and enzymes of carotenoid biosynthesis in plants. *Annu Rev Plant Physiol Plant Mol Biol*. 1998;49:557-583.
5. Misawa N. Pathway engineering for functional isoprenoids. *Curr Opin Biotechnol*. 2011;22(5):627-633.
6. Bjerkeng B. Carotenoid pigmentation of salmonid fishes. *Recent Adv Mar Biotechnol*. 2000;4:71-90.
7. Higuera-Ciupara I, Felix-Valenzuela L, Goycoolea FM. Astaxanthin: a review of its chemistry and applications. *Crit Rev Food Sci Nutr*. 2006;46(2):185-196.
8. Østerlie M, Bjerkeng B, Liaaen-Jensen S. Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration. *J Nutr Biochem*. 2000;11(10):482-490.
9. Shah MMR, Liang Y, Cheng JJ, Daroch M. Astaxanthin-producing green microalga *Haematococcus pluvialis*: from single cell to high value commercial products. *Front Plant Sci*. 2016;7:531.
10. Leclercq E, Taylor JF, Hunter D, Migaud H. Body size dimorphism of sea-reared Atlantic salmon. *Aquac Int*. 2010;18:1077-1091.
11. NRC. Nutrient Requirements of Fish and Shrimp. National Academies Press. 2011:1-376.
12. Tolasa S, Cakli S, Ostermeyer U. Determination of astaxanthin and canthaxanthin in salmonid. *Eur Food Res Technol*. 2005;221(6):787-791.
13. Ytrestøl T, Bjerkeng B. Dose response in uptake and deposition of intraperitoneally administered astaxanthin in Atlantic salmon. *Aquaculture*. 2007;263(1-4):179-191.
14. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR. *Methods*. 2001;25(4):402-408.
15. Storebakken T, Foss P, Schiedt K, Austreng E, Liaaen-Jensen S, Manz U. Carotenoids in diets for salmonids: pigmentation of rainbow trout with synthetic astaxanthin. *Aquaculture*. 1987;65(3-4):279-292.
16. Sommer TR, Potts WT, Morrissy NM. Utilization of microalgal astaxanthin by rainbow trout. *Aquaculture*. 1991;94(1):79-88.