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Unlocking the Nutritional Potential of Endemic Salmonid Species (Black Sea Salmon, *Salmo labrax*): Carotenoids and Their Impact on Fillet Characteristics

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Abstract

This study evaluated the fillet quality of Black Sea salmon following diets supplemented with carotenoids such as astaxanthin, canthaxanthin, and lycopene. Carotenoid supplementation improved crude protein, essential amino acids, and unsaturated fatty acids. Astaxanthin and canthaxanthin increased crude fat and texture, while lycopene reduced these values. Lycopene-fed fish exhibited the highest polyunsaturated/saturated fatty acid ratio and improved health indices, including fish lipid quality (FLQ) and health-promoting index (HPI). The highest PUFA/SFA ratios were observed at 200–300 ppm lycopene. Amino acid scores also improved, with canthaxanthin elevating the digestible indispensable amino acid score (DIAAS) of young children, categorizing fillets as excellent protein sources. In older age groups, lycopene at 100 ppm yielded the highest DIAAS scores. Notably, carotenoid-supplemented fillets contained higher levels of glycine, alanine, and omega-3 fatty acids (EPA, DHA), supporting muscle structure and cardiovascular health. Lycopene emerged as a potent antioxidant, demonstrating untapped potential for aquaculture applications. These findings underline the multifaceted benefits of carotenoids in enhancing meat and lipid quality, emphasizing the need for future research on optimized supplementation strategies to maximize both health benefits and aquaculture performance.

Introduction

Black Sea salmon is an endemic fish species, predominantly distributed on the northeastern coasts of the Black Sea and basins of the Azov and Caspian Seas (Okumuş et al., 2004). However, the natural stocks of this species have been endangered, mainly due to the pressure of overfishing (Çakmak et al., 2022). The fall in the natural stocks of Black Sea salmon has led researchers to cultivate the species, and preliminary studies started in 1998 with the adaptation trials (Çakmak et al., 2019). Black Sea salmon is extensively cultivated today, resulting in culture characteristics and meat yield enhanced through selectivity programs over the years (Kasapoglu et al., 2020; Özel et al., 2023). Despite economic value of the species, consumers expect the fillet color of Black Sea salmon to be similar to other commercial salmon species, typically red or pink tones (Çankırılıgil et al., 2022).

Fillet pigmentation is crucial in seafood quality, dramatically influencing consumer preferences (García-Chavarría & Lara-Flores 2013). Salmonids have a unique reddish/pinkish fillet color in their natural habitat mainly because of their diet, consisting of carotenoid-rich species like algae and zooplankton (Molkentin et al., 2015; Nakano & Wiegertjes 2020). However, in farmed salmons, required carotenoids cannot be obtained with traditional fish feed, and dietary carotenoids are used as feed additives to achieve meat and skin pigmentation (Landry et al., 2024). Astaxanthin and canthaxanthin are the most commonly used carotenoids for fillet pigmentation, contributing to salmon's commercial

value (Buttle et al., 2001; Landry et al., 2024). Likewise, lycopene is another beneficial carotenoid found mainly in tomato products and marine algae (Costa-Rodrigues et al., 2018). Lycopene is generally known for its health-promoting properties, having a high singlet oxygen quenching rate and potent antioxidant activity owing to its apolar and acyclic structure (Tang et al., 2015), but it also contributes to pigmentation in some fish species (Sahin et al., 2014).

Marine carotenoids are also beneficial bioactive substances with several health-promoting effects (Page & Davies 2006; Steven et al., 2022). They slow cellular degradation and oxidative stress (Genç et al., 2020; Nakano & Wiegertjes 2020), improve the immune system, and prevent neurodegenerative diseases (Genç et al., 2020) in metabolism with their antioxidant properties. With such benefits, carotenoids ensure fillet pigmentation and simultaneously contribute to fish growth and development (Page & Davies, 2006). Thus, carotenoid supplementation in aquaculture is indispensable, especially for species feeding carotenoid-rich sources in their natural habitat, like salmonids.

In the previous study, Black Sea salmon were fed with different carotenoid-supplemented feeds, and the impacts of astaxanthin, canthaxanthin, and lycopene additives were evaluated. The most effective pigmentation was determined in fish fed with 300 ppm astaxanthin for 90 days, while all three carotenoid additives positively affected the growth parameters of the fish. On the other hand, the effects of all three carotenoid groups on meat yield, weight gain, and sensory characteristics of fish were found to be statistically different. Black Sea salmon individuals' meat yields and weight gains increased by astaxanthin and canthaxanthin. However, while these parameters decreased in the lycopene group, the highest length gain was detected in the same group. Also, panelists reported that while meat hardness and oiliness values decreased in the lycopene group, they increased in the canthaxanthin group (Çankırılıgil et al., 2022). We think that differences in growth parameters and sensory characteristics may be the consequence of chemical changes in the fish fillet, considering each carotene has a different impact on the metabolism of Black Sea salmon. These findings align with a study by Lerfall et al., (2016), which reported a positive correlation between fillet pigmentation and polyunsaturated fatty acid content in Atlantic salmon fed with carotene-heavy diets.

The study aims to investigate the effects of carotenoids, which have become increasingly prevalent in feeding Black Sea salmon, on nutrient content and texture parameters. By examining these aspects, the study seeks to gain insights into the potential changes in meat quality and understand the metabolic impacts of various carotenoids on Black Sea salmon. In light of this, we hypothesize that the dietary supplementation of carotenoids in Black Sea salmon will significantly impact the fish fillets' nutrient content and texture parameters.

Material and Methods

Fish Material and Production Process

Black Sea salmon (*Salmo labrax*) individuals were obtained from a project titled "A Research on Possibilities of Using Some Phytobiotic Containing Diets in Black Sea Salmon Nutrition". These fish were fed with diets containing carotenoids, including astaxanthin, canthaxanthin, and lycopene, at graded levels of 100, 200, and 300 ppm. The control group was fed a carotenoid-free diet. In all trials, fish individuals were fed daily at a rate equivalent to 2% of their body weight for 90 days in open-system fiber tanks and seawater conditions (15°C and 18‰ salinity). In total, 400 fish individuals weighing about 300 g each were obtained from this study for use in the present research. A graphical illustration of the study is shown in Figure 1.

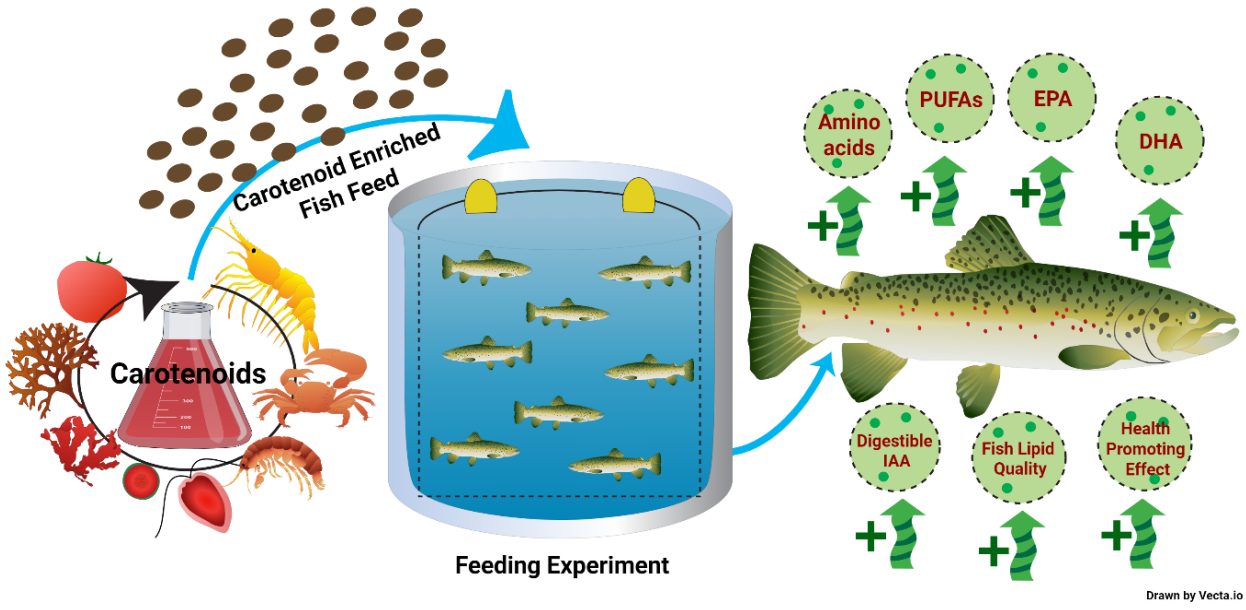

Figure 1. Graphical illustration of the study. Image was drawn with Vecta.io (2024).

The diets used to feed the fish contained fish meal (33%) (European sprat and Atlantic herring, 50:50), soybean meal (16%), green pea protein (12%), wheat meal (11.5%), sunflower meal (7%), wheat gluten (6%), fish oil (14%) (European anchovy), multivitamins (0.34%), multimineral (0.15%), ascorbic acid (0.1%), and alpha-tocopherol (0.04%). The multivitamin mix included 300 mg Myo-inositol, 200 mg biotin, 50 mg calcium pantothenate, 30 mg riboflavin, 20 mg pyridoxine, 20 mg thiamine, 12 mg menadione, 6 mg niacin, 0.6 mg retinol, 0.5 mg folic acid, 0.05 mg cholecalciferol, and 0.05 mg cobalamin. The multimineral mix contained 50 mg ferric sulfate heptahydrate, 50 mg manganese (II) oxide, 50 mg zinc oxide, 10 mg copper sulfate pentahydrate, 0.8 mg calcium iodate, 0.15 mg cobalt carbonate hexahydrate, and 0.15 mg sodium selenite (Çankırılıgil et al., 2022). Astaxanthin standard (≥98% pure, HPLC grade, Sigma-Aldrich A3236), canthaxanthin standard (≥95% pure, HPLC grade, Supelco 32993), and lycopene standard (≥85% pure, HPLC grade, Supelco 75051) were used for fillet pigmentation and were incorporated into the diets at graded levels of 100, 200, and 300 ppm.

The fish obtained from the feeding trials were anesthetized using clove oil baths (50 $\mu L/L^{-1}$) and slaughtered by gill cutting in accordance with ARRIVE guidelines and EU directives for animal experiments (European Commission 2010; Kilkenny et al., 2010). Subsequently, internal organs were removed, and the fish were skinned and filleted. The fillets were set aside for texture analysis. Following this, all fillets were homogenized and stored for chemical analyses.

Texture Analysis

Texture analysis was performed according to Schubring's (2002) study. In this method, a texture analyzer with a 5 kg load cell (Texture Technologies, TA.XT Plus) was used to apply pressure to determine the texture properties of fish fillets. A flat cylindrical probe (75 mm diameter) was used. Texture profile analysis (TPA) was executed with uniform cross-head speeds of 1.0 mm/s^{-1} during the pre-test, test, and post-test stages. Samples were compressed to 50% of their initial height in two successive cycles, activated by a 5 g trigger load. The analyses were carried out in TPA mode, and the fish meat's hardness, adhesiveness, flexibility, cohesiveness, gumminess, chewiness, and resilience values were determined. All analyses were conducted in five parallel, and the obtained peaks were interpreted as g-force.

Determination of Proximate Composition

Moisture was determined using the Horwitz method specified in AOAC (2000). After the samples were weighed into tared petri plates, they were dried for approximately 6 hours in a 100°C oven. The petri plates were cooled by soaking in the desiccator for 30 minutes, weighed on a precision balance, and the obtained value was calculated as a percentage. Crude protein was evaluated according to the Kjeldahl method specified in AOAC (2000). 1 g of the samples was weighed and digested at 450°C with 15 ml H_2SO_4 and a selenium-containing Kjeldahl tablet. After the obtained hydrolysates were diluted with distilled water (1:2), they were distilled with NaOH in the distillation unit. The distilled samples were titrated with 0.1 N HCl, and the obtained data were calculated by multiplying the protein factor (6.25) indicated for the fish. Crude fat analysis was carried out according to Folch et al. (1957). Accordingly, 10 ml of methanol-chloroform solution was added to the samples, which weighed 5 g in tared balloons. The samples were then kept in a light-free compartment for 12 hours. In the following, the samples were filtered through a 1.2 µm filter and condensed in an extractor with a temperature fixed at 60°C. The crude fat ratio was calculated by weighing the balloons containing concentrated samples. Crude ash analysis was performed using the Horwitz method in AOAC (2000). 1 g of the samples weighed in the tare porcelain crucibles were burned in the ash oven at 600°C for about 6 hours till the samples turned into white ash. Then, the cooled crucibles were kept in the desiccator for 30 minutes. The final weights were calculated as a percentage.

Amino Acid Analysis

Pre-treatment of amino acid analyses was carried out using Çankırılıgil et al. (2020)'s method. Acidic digestion was applied to the samples as a pre-treatment. Samples were entirely hydrolyzed with HCl for about 24 hours at 110 °C. The obtained hydrolysates were filtered through a 0.45 µm PTFE syringe filter and diluted in a ratio of 10^{-1} with distilled water. Filtrates were stored in 2 mL amber vials until the analyses were conducted. Amino acid analyses were performed according to Henderson et al. (2000) by Agilent HPLC Infinity II system having a DAD detector and auto-sampler. In analysis, derivatization was performed automatically by an autosampler with borate, OPA, and FMOC reagents. Following the process, samples were injected into the HPLC system. Each sample was analyzed five times (n:5). Amino-acid separations were conducted on a Zorbax Eclipse AAA column employed as the stationary phase. Eluent A comprised 40 mM Na_2HPO_4 buffer, whereas eluent B was a 45:45:10 (v/v/v) methanol–acetonitrile–water mixture brought to pH 7.8 with 10 N NaOH. The gradient programme proceeded as follows: in 1.9 min, A:B=100:0%; in 18.1 min, A:B=43:57%; in 18.6 min A:B=0:100% (held to 22.3 min); and in 23.2 min A:B=100:0%. Chromatography was run at 40°C with 2 mL/min flow rate. Amino acids bound to the OPA reagent were detected at 338 nm, and those bound to the FMOC reagent were detected at 262 nm. The peaks obtained were calibrated and compared with their standard curves.

Determination of Amino Acid Quality

To assess the amino acid quality of Black Sea salmon fillets fed with carotenoid-enriched diets, the digestible indispensable amino acid score (DIAAS) method was employed (FAO 2013). This method involves scoring the contents of indispensable amino acids, accounting for their digestibility and considering daily human amino acid requirements (Kendler et al., 2023). Initially, each essential amino acid (IAA) was quantified and reported in milligrams per gram of protein (mg g^{-1} protein). Subsequently, the digestible IAA reference ratios (DIAA) were computed by multiplying the true ileal digestibility coefficients (df) for the respective indispensable amino acid by the grams of protein in the samples (FAO 2013; Kendler et al., 2023).

$$\text{mg amino acid per g protein} = \frac{\text{mg amino acid per g wet weight}}{\text{g protein per g wet weight}}$$

$$\text{Digestible IAA reference ratio} = \frac{\text{mg of amino acid in 1 g sample protein x (df/100)}}{\text{mg of amino acid in 1 g of reference protein}}$$

True ileal digestibility reflects the recovery and losses of amino acids from specific protein sources during the digestive process. Due to limited digestibility data for particular fish species, including Black Sea salmon, Moughan et al. (2012) provided amino acid digestibility values for fish muscle in general. These values were subsequently utilized by Shaheen et al. (2016) to evaluate the digestibility of various fish species, such as *Pangasius pangasius* (pangas), *Labeo rohita* (rohu), and *Oreochromis mossambicus* (tilapia) meats. In this study, true ileal digestibility factors for fish-based amino acids were adopted as follows: 85% for histidine, 93% for isoleucine, 91% for leucine, 93% for lysine, 83% for phenylalanine, 95% for threonine, and 90% for valine. In the absence of specific digestibility data for cysteine, methionine, and tyrosine, the true ileal digestibility of the fish protein was fixed at 90 % (Moughan et al., 2012; Shaheen et al., 2016).

Reference protein represents daily amino acid requirements outlined by WHO/FAO/UNU (2007) for three distinct age groups: infants (birth to 6 months), children (6 months to 3 years), and older children, adolescents, and adults. DIAA reference ratios for infants were excluded from calculations as fish are generally not introduced to infants before six months (Kull et al., 2006). Finally, Following FAO (2013) guidelines, the smallest digestible indispensable amino acid ratio (DIAA) was multiplied by a factor of 100, thereby generating the DIAA score (DIAAS) for each sample.

$$\text{DIAAS \%} = 100 \times \text{lowest digestible IAA reference ratio of given amino acids}$$

Fatty Acid Analysis

Fatty acid analysis was performed according to IUPAC (1979). An aliquot of lipid (0.15 g), obtained during the crude-fat extraction, was placed in a round-bottom flask together with 5 mL of 0.5 N methanolic NaOH and a boiling chip. Saponification proceeded at 65°C for 15 min. Subsequently, 5 mL of boron-trifluoride–methanol reagent was added, and esterification continued at the same temperature for 2 min. After cooling, 2 mL of heptane was introduced, and the mixture stood for 1 min to extract the fatty-acid methyl esters (FAMEs). The organic layer was separated by washing with saturated NaCl solution, filtered through a 0.45 µm syringe filter, and transferred to autosampler vials. FAMEs were analyzed on a Shimadzu GC-17A with a flame-ionization detector and a 50 m capillary column. The oven program was initiated at 140°C, ramped at 4°C per minute to 240°C, and was then held isothermally. Injector and detector temperatures were maintained at 260°C. Helium (30 mL/min) served as the carrier gas, while hydrogen (40 mL/min) and air (400 mL/min) supplied the detector; the total flow was 22.8 mL/min.

Calculation of Lipid Quality Indices

To assess nutritional quality changes in salmon fillets associated with feeding experiments, lipid quality indices, including the polyunsaturated fatty acid/saturated fatty acid ratio (PUFA/SFA), linoleic acid/α-linolenic acid ratio (LA/ALA), fish lipid quality (FLQ), health-promoting index (HPI), atherogenicity index (AI), thrombogenicity index (TI), hypo/hypercholesterolemic ratio (h/H), and unsaturation index (UI), were calculated. The methodologies described by Chen & Liu (2020) for PUFA/SFA, LA/ALA, FLQ, HPI, UI, Santos-Silva et al. (2002) for h/H, Ulbricht & Southgate (1991) for AI, and TI were used.

$$PUFA/SFA = \sum PUFA \,/\, \sum SFA$$

$$LA/ALA = C18{:}2\ n-6 \,/\, C18{:}3\ n-3$$

$$FLQ = 100 \times (C22{:}6\ n-3 + C20{:}5\ n-3) / \sum FA$$

$$HPI = \sum UFA \,/\, [C12{:}0 + (4 \times C14{:}0) + C16{:}0]$$

$$UI = 1 \times (\%\text{monoenoics}) + 2 \times (\%\text{dienoics}) + 3 \times (\%\text{trienoics}) + 4 \times (\%\text{tetraenoics}) + 5 \times (\%\text{pentaenoics}) + 6 \times (\%\text{hexaenoics})$$

$$HH = (C18{:}1 + \sum PUFA) \,/\, (C14{:}0 + C16{:}0)$$

$$AI = \frac{[\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}]}{(\sum \text{MUFA} + \sum \text{PUFA})}$$

$$TI = \frac{(\text{C14:0} + \text{C16:0} + \text{C18:0})}{[(0.5 \times \sum \text{MUFA}) + (0.5 \times \sum \text{n-6 PUFA}) + (3 \times \text{n-3 PUFA}) + (\text{n-3} / \text{n-6})]}$$

FA represents total fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids.

Data Evaluation

Differences in chemical composition and texture data were evaluated using one-way analysis of variance (ANOVA). Variance homogeneity and normality were verified with Levene's and Anderson–Darling tests, respectively. Results were expressed as mean value±standard error. The significance level (P value) was set as 0.05, and mean values less than 0.05 indicate they are significant. In all statistical analyses, the IBM SPPS package program was used.

Result and Discussion

Texture

Fish flesh quality can be shaped by numerous biotic and abiotic variables—species, sex, body size, age, reproductive phase, and environmental temperature among them (Nurnadia et al., 2011). In the present experiment, all specimens were reared under constant conditions, ensuring the same age and comparable length and weight across groups. This ensured that the salmon individuals were homogeneous and exhibited no initial differences in meat quality.

In salmonids, 90% of the striated muscles are located on both sides of the fish from head to tail in white muscles bundles called myotomes (Kiessling et al., 2006). These white muscles are found to be interwoven large and small fibers, forming the appearance of a mosaic muscle structure specific to salmonids and directly affecting the texture properties (Valente et al., 1999). The texture is an essential quality parameter in fulfilling consumer expectations (Johnston et al., 2006), and the hardness parameter is important for salmonid species (Mørkøre et al., 2009). Moreno et al., (2012) reported that salmonids having firmer flesh closely resembled natural characteristics. In addition, muscle tissue that is not firm enough in salmonids cannot be evaluated effectively and causes economic losses (Torgersen et al., 2014). The present study's findings indicate that hardness values increased and peaked after the three-month trial in the astaxanthin and canthaxanthin groups. Conversely, the lycopene-supplemented groups showed a decrease in meat hardness throughout the trial period, and the hardness values were statistically lower relative to the control (P≤0.05). The highest hardness among the groups was determined in 300 ppm, 200 ppm, and 100 ppm astaxanthin groups with 1170.64±37.76 g, 1096.42±31.76 g, 1073.52±35.13 g, along with 300 ppm canthaxanthin group with 1091.98±17.20. The lowest hardness values were detected in lycopene groups as 462.26±23.66 g for 300 ppm, 524.97±17.26 g for 200 ppm, and 534.71±11.83 g for 100 ppm. In all groups fed with astaxanthin and canthaxanthin, the gumminess parameter increased along with hardness, while all other parameters decreased with the increasing amount of carotenoids. On the other hand, the lycopene-fed groups experienced a decrease in hardness, and the different parameters increased with the increase in the carotenoid amounts. The increase in hardness observed in astaxanthin- and canthaxanthin-fed groups might be associated with their antioxidant properties that stabilize muscle proteins and enhance the integrity of connective tissue structures. These carotenoids could reduce oxidative damage in muscle fibers, thereby leading to firmer meat texture. In contrast, lycopene, although a potent antioxidant, may promote different metabolic pathways affecting connective tissue and muscle composition, resulting in softer fillet textures. Results are shown in Figure 2, and raw data of the analyses are shown in Supplementary Information.

Proximate Composition

The striated muscles in fish meat consist of primary sections: contractile proteins, lipids, and connective tissue, and changes in the quantity of these sections affect meat quality (Kiessling et al., 2006). According to the results, while an increase in moisture and crude ash amounts was detected in the astaxanthin and lycopene groups, a statistical decrease was determined in the moisture and crude ash contents of the canthaxanthin group by the conclusion of the trial (P≤0.05). The proximate composition of Black Sea salmon fillets shown in Table 1. It was reported that the increased amount of water in muscle tissue affects texture by increasing meat juiciness (Dunajski 1979). In fish meat, meat tightness rises with water on the fish muscle (Hyldig & Nielsen 2001). According to the texture results, the fish muscle has a higher hardness value in the 90th-day control group compared to initial values (P≤0.05). This is because salmonids show myotome development with growth, thus increasing meat hardness (Kiessling et al., 2006; Moreno et al., 2012). The crude protein and lipid contents of Black Sea salmon fed with astaxanthin and canthaxanthin-based diets increased with the carotenoid dosage. However, the crude protein content increased in lycopene groups, while the crude fat content decreased statistically with the rising lycopene amount (P≤0.05). At the end of the trial, the highest crude fat was determined in the 300 ppm canthaxanthin group, while the lowest ratio was found in the 300-ppm lycopene group (P≤0.05). Although protein content increased in all groups at the end of the trial, no statistical differences were detected. The increase in the protein ratio of the fish after three months of the feeding trial can be explained by the muscle development that occurs with the growth of the fish. In our study, the fish feed used has 18% crude fat. For this reason, fat accumulation was expected in fish meat during the trial period. However, at the trial's end,

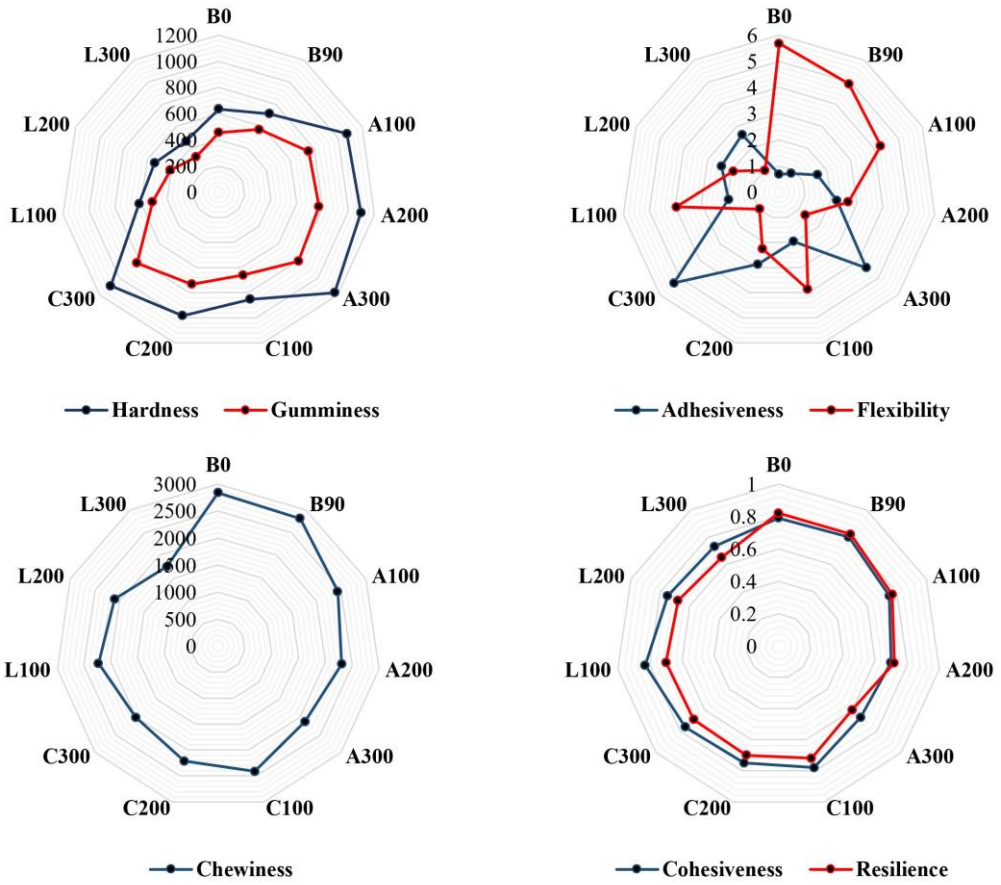

Figure 2. Textural properties of Black Sea salmon fillets at the end of 90-day feeding trials based on carotenoid supplementation. Basal diet-fed groups in the initial (0) and the last day (90) of the feeding trials were described as B0 and B90, respectively. For each carotenoid group, 100ppm, 200ppm, and 300ppm carotenoid-containing diets expressed as A100, A200, and A300 for astaxanthin, C100, C200, and C300 for canthaxanthin, L100, L200, and L300 for lycopene, respectively.

Table 1. Proximate composition of Black Sea salmon fillets at the end of 90-day feeding trials

		Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Basal Diet	Day 0	73.66±0.18^{f}	17.58±0.17^{b}	5.84±0.24^{b}	1.41±0.02^{b}
	Day 90	72.84±0.52^{ab}	17.72±0.14^{ab}	6.70±0.25^{a}	1.40±0.04^{ab}
Astaxanthin	100 ppm	73.49±0.41^{a}	17.74±0.18^{ab}	6.72±0.15^{a}	1.42±0.07^{a}
	200 ppm	73.17±0.12^{a}	17.82±0.21^{ab}	6.79±0.17^{a}	1.40±0.05^{ab}
	300 ppm	73.02±0.38^{a}	18.01±0.10^{a}	6.79±0.19^{a}	1.45±0.04^{a}
Canthaxanthin	100 ppm	72.59±0.82^{a}	17.89±0.15^{ab}	7.15±0.23^{c}	1.33±0.06^{b}
	200 ppm	71.26±0.29^{bc}	18.08±0.21^{a}	8.14±0.11^{a}	1.25±0.03^{c}
	300 ppm	71.18±0.41^{bc}	18.26±0.18^{a}	8.25±0.18^{a}	1.25±0.03^{c}
Lycopene	100 ppm	74.33±0.11^{c}	17.82±0.26^{a}	4.29±0.14^{e}	1.50±0.05^{a}
	200 ppm	74.73±0.23^{b}	18.08±0.14^{a}	4.27±0.11^{e}	1.52±0.03^{a}
	300 ppm	75.18±0.14^{a}	18.08±0.23^{a}	4.19±0.16^{e}	1.55±0.03^{a}

Values expressed as mean value ± standard error and different superscripts in a column represent statistical differences, and the p-value is accepted as 0.05.

crude fat was decreased in the lycopene group. Some studies show that tomato products and additives containing lycopene reduce fat accumulation in humans, which can cause their anti-obesity properties. Lycopene-enriched tomato wine has been reported to affect weight control by influencing the lipid enzyme metabolism in mice (Kim et al., 2012). Another study about patients who regularly consumed tomato juice daily reported that waist circumferences were thinned, and weight control was achieved (Li et al., 2015). Considering similar studies, it can be suggested that crude fat decreased due to the metabolic effects of lycopene. Lycopene, a powerful antioxidant, causes many reactions in living metabolism (Hossain 2011; Kulawik et al., 2023). Despite all these studies, the relationship between lycopene intake and fat burning in humans and other animals has not been fully proven. Scientific studies on the use of lycopene, especially in fish, are pretty scarce. For this reason, considering the results of these studies, further scientific studies on using lycopene as a feed additive are needed.

Amino Acids

White muscle tissue (myotome), which makes up a large part of the amino acids in fish, is highly susceptible to autooxidation (Ballantyne 2011). Carotenoids, potent antioxidants, are assumed to reduce amino acid autooxidation as their accumulation in muscle tissue increases. According to the results, total amino acid levels elevated significantly in every group by the conclusion of the trial (P≤0.05). The highest amino acids in the Black Sea salmon muscle are leucine, serine, glutamic acid, and lysine (P≤0.05). The amino acid composition of Black Sea salmon at the end of the trials is shown in Table 2. Seven essential amino acids, namely threonine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine, were detected in all groups, along with histidine, which is semi-essential. Tryptophan was also detected, but the contents were slightly above the detection limit, between 0.01±0.01 and 0.03±0.02 g/100g, with high standard error. Tryptophan is vulnerable to low pH and high heat and can be lost entirely during acidic digestion (Cuq & Friedman 1989). Thus, due to statistical incoherency of the tryptophan data caused by acidic pre-treatment, tryptophan was not evaluated in this study. Similarly, glutamine and asparagine can become glutamic and aspartic acids, respectively (Moreno et al., 2012). Thus, glutamic acid was found to be the highest. Glycine and alanine, which contribute to the hexagonal muscle shape unique to salmonids, may be more common in fish with firmer meat texture. Similarly, salmonids with softer meat have higher amounts of threonine, valine, isoleucine, and leucine (Moreno et al., 2012). Our results were similar to the cultured *Salmo trutta* sp. findings of Kaya et al. (2014). According to the study results, the amount of glycine and alanine were higher in fish on astaxanthin- and canthaxanthin-supplemented diets (P≤0.05). At the same time, no statistical differences were found in lycopene groups (P≥0.05). Similarly, lycopene trials increased amounts of threonine, isoleucine, and valine in fish meat (P≤0.05). In astaxanthin trials, valine and leucine decreased, while threonine and isoleucine increased. Finally, in canthaxanthin groups, threonine and leucine amounts decreased, but isoleucine increased (P≤0.05). Considering these results, it can be seen that the changes in meat texture are associated with the amino-acid profile and the proximate composition changes mentioned above. These results are parallel to the results of texture analysis. The observed differences in amino acid compositions among groups could be attributed to the distinct antioxidant capacities and metabolic influences of each carotenoid. Astaxanthin and canthaxanthin may support the synthesis and protection of structural muscle proteins, leading to higher levels of glycine and alanine that are associated with firmer muscle texture. Meanwhile, lycopene supplementation might modulate amino acid metabolism differently, promoting the accumulation of threonine, valine, and isoleucine, which are typically higher in softer textured fish.

The DIAAS values for Black Sea salmon fillets are shown in Table 3, while the individual indispensable amino acid (IAA) compositions are depicted in Figure 3 and detailed in the appendices [Supplementary Information (SI)]. The quality of a protein is often determined using the digestible indispensable amino acid score (DIAAS), where a rating higher than 100 signifies an excellent protein source, a score between 75-100 is considered good, and a score lower than 75 is categorized as low (FAO 2013). Based on our findings, the DIAAS scoring pattern for young children revealed valine as the limiting amino acid in the canthaxanthin group, while aromatic amino acids (AAA) were limiting for the remaining groups. Notably, only the canthaxanthin groups, specifically those with 200 ppm and 300 ppm, achieved DIAAS values surpassing the basal groups, registering 100. According to FAO (2013), these latter two groups can be classified as excellent protein sources. Interestingly, canthaxanthin supplementation exceeding 200 ppm was observed to increase the phenylalanine and tyrosine contents (Table 2) of the muscle, originally measured as AAA in the control groups. Conversely, astaxanthin supplementation beyond 200 ppm resulted in a reduction of phenylalanine, leading to DIAAS scores falling below 75, considered sublimit on the scale for a good protein source. Despite utilizing the same amino acid data for calculations, distinct amino acid patterns emerged for adults. In the DIAAS calculation for older children, adolescents, and adults, all nine trial groups, including the basal ones, exhibited DIAAS values exceeding 100, categorizing them as excellent protein sources. Valine emerged as the limiting amino acid for the control groups, lycopene groups, and the 100-ppm astaxanthin group, while AAA posed limitations for canthaxanthin groups and the 200-ppm and 300-ppm astaxanthin groups. The latter two astaxanthin groups yielded DIAAS values of 95 and 90, respectively, owing to a decline in phenylalanine and valine (Table 2). Notably, the 100-ppm lycopene group exhibited the highest DIAAS score of 111, surpassing even the control groups. Interestingly, despite carotenoid supplementation increasing total amino acids, these effects did not translate to observable changes in DIAAS calculations.

Fatty Acids

The highest saturated fatty acid in all groups was palmitic acid, the monounsaturated fatty acids were oleic acid and palmitoleic acid, and polyunsaturated fatty acids were DHA, linoleic acid, and EPA, respectively. Linoleic acid and alpha-linolenic acid, which were essential for humans, were identified in all groups. While total monounsaturated fatty acids (MUFA) and total polyunsaturated fatty acids (PUFA) increased (P≤0.05), the total saturated fatty acids (SFA)

Table 2. Amino acid composition of Black Sea salmon fillets at the end of 90-day feeding trials (g/100g)

Amino	Basal Diet		Astaxanthin			Canthaxanthin			Lycopene		
Acids	Day 0	Day 90	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm
Asp	0.84±0.04^{a}	0.88±0.02^{a}	0.83±0.03^{ab}	0.71±0.02^{c}	0.75±0.02^{cb}	0.88±0.02^{a}	0.82±0.03^{ab}	0.78±0.04^{b}	0.82±0.03^{ab}	0.76±0.02^{b}	0.76±0.02^{b}
Glu	1.54±0.06^{b}	1.53±0.02^{b}	1.67±0.07^{b}	1.74±0.03^{ab}	1.80±0.03^{ab}	1.64±0.07^{b}	1.70±0.09^{ab}	1.90±0.16^{a}	1.58±0.05^{b}	1.62±0.03^{b}	1.69±0.05^{ab}
Asn	0.33±0.02^{c}	0.34±0.01^{c}	0.65±0.04^{ab}	0.62±0.05^{b}	0.63±0.04^{b}	0.62±0.03^{b}	0.60±0.02^{b}	0.69±0.02^{ab}	0.63±0.03^{b}	0.65±0.04^{ab}	0.72±0.03^{a}
Ser	1.55±0.04^{bc}	1.51±0.02^{c}	1.51±0.04^{c}	1.37±0.06^{d}	1.34±0.07^{d}	1.65±0.04^{b}	1.81±0.06^{a}	1.86±0.09^{a}	1.62±0.05^{b}	1.72±0.07^{ab}	1.83±0.08^{a}
Gln	0.59±0.03^{d}	0.62±0.02^{cd}	0.75±0.05^{bc}	0.86±0.05^{ab}	0.98±0.10^{a}	0.79±0.03^{b}	0.78±0.02^{b}	0.86±0.04^{ab}	0.66±0.04^{c}	0.75±0.03^{bc}	0.81±0.03^{b}
His	0.63±0.02^{ab}	0.71±0.03^{a}	0.64±0.04^{ab}	0.58±0.03^{b}	0.67±0.04^{a}	0.78±0.03^{a}	0.65±0.03^{b}	0.74±0.03^{a}	0.76±0.04^{a}	0.77±0.04^{a}	0.79±0.03^{a}
Gly	0.63±0.01^{d}	0.67±0.02^{c}	0.69±0.02^{c}	0.72±0.02^{b}	0.81±0.03^{a}	0.72±0.04^{b}	0.74±0.03^{b}	0.75±0.02^{b}	0.67±0.02^{c}	0.66±0.02^{c}	0.68±0.03^{c}
Thr	0.84±0.04^{c}	0.81±0.04^{c}	1.14±0.08^{ab}	1.19±0.10^{ab}	1.33±0.09^{a}	0.72±0.03^{d}	0.73±0.04^{d}	0.71±0.04^{d}	0.98±0.07^{b}	1.10±0.08^{ab}	1.13±0.09^{ab}
Ala	0.31±0.01^{b}	0.30±0.01^{b}	0.32±0.02^{b}	0.36±0.03^{ab}	0.40±0.02^{a}	0.34±0.01^{b}	0.40±0.01^{a}	0.42±0.02^{a}	0.31±0.03^{b}	0.30±0.02^{b}	0.31±0.02^{b}
Tyr	0.16±0.01^{c}	0.18±0.02^{b}	0.16±0.03^{c}	0.15±0.01^{c}	0.15±0.02^{c}	0.18±0.02^{ab}	0.21±0.01^{a}	0.19±0.02^{ab}	0.18±0.01a^{b}	0.19±0.01a^{b}	0.23±0.02^{a}
Cys	0.13±0.01^{ab}	0.13±0.01^{ab}	0.13±0.02^{ab}	0.13±0.03^{ab}	0.15±0.04^{a}	0.14±0.01^{ab}	0.14±0.01^{ab}	0.15±0.02^{a}	0.12±0.01^{b}	0.14±0.01^{ab}	0.15±0.01^{a}
Val	0.86±0.02^{ab}	0.87±0.04^{ab}	0.79±0.03^{b}	0.75±0.05^{b}	0.76±0.05^{b}	0.85±0.04^{ab}	0.86±0.06^{ab}	0.87±0.09^{a}	0.98±0.06^{a}	0.94±0.04^{a}	0.96±0.04^{a}
Met	0.65±0.03^{c}	0.68±0.02^{c}	0.78±0.02^{b}	0.80±0.04^{ab}	0.89±0.04^{a}	0.65±0.03^{c}	0.65±0.02^{c}	0.65±0.04^{c}	0.75±0.03^{b}	0.80±0.05^{ab}	0.83±0.04^{a}
Phe	0.86±0.03^{ab}	0.85±0.03^{ab}	0.78±0.03^{b}	0.67±0.03^{c}	0.64±0.04^{c}	0.94±0.08^{a}	0.93±0.07^{a}	0.96±0.09^{a}	0.78±0.04^{b}	0.69±0.02^{c}	0.64±0.04^{c}
Ile	0.74±0.06^{c}	0.85±0.03^{b}	0.90±0.02^{ab}	0.97±0.03^{a}	0.95±0.02^{a}	0.89±0.06^{ab}	0.89±0.06^{ab}	0.93±0.04^{a}	0.93±0.05^{a}	0.98±0.04^{a}	1.00±0.05^{a}
Leu	1.83±0.07^{a}	1.68±0.09^{ab}	1.58±0.03^{bc}	1.52±0.04^{bc}	1.49±0.04^{c}	1.77±0.07^{a}	1.74±0.11^{a}	1.76±0.12^{a}	1.64±0.05^{b}	1.60±0.06^{b}	1.61±0.05^{b}
Lys	1.13±0.06^{c}	1.34±0.08^{b}	1.30±0.04^{b}	1.41±0.04^{a}	1.47±0.08^{a}	1.38±0.11^{ab}	1.41±0.08^{a}	1.46±0.11^{a}	1.35±0.03^{b}	1.44±0.05^{a}	1.46±0.05^{a}
∑AA	13.62±0.12^{e}	13.96±0.12^{d}	14.62±0.13^{c}	14.55±0.17^{c}	15.21±0.25^{a}	14.98±0.19^{b}	15.06±0.17^{b}	15.70±0.20^{a}	14.77±0.13^{bc}	15.11±0.17^{b}	15.62±0.22^{a}

Values expressed as mean value ± standard error and different superscripts in a line represent statistical differences, and the p-value is accepted as 0.05. Asp: aspartic acid, Glu: glutamic acid, Asn: asparagine, Ser: serine, Gln: glutamine, His: histidine, Gly: glycine, Thr: threonine, Ala: alanine, Tyr: tyrosine, Cys: cysteine, Val: valine, Met: methionine, Phe: phenylalanine, Ile: isoleucine, Leu: leucine, Lys: lysine, ∑AA: total amino acids.

Table 3. Digestible indispensable amino acid reference ratios (DIAA) and DIAA score (DIAAS) for Black Sea salmon fillets at the end of 90-day feeding trials

	Basal Diet		Astaxanthin			Canthaxanthin			Lycopene		
	Day 0	Day 90	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm
DIAA calculations for young children (6 months to 3 years old)*											
AAA	0.94	0.94	0.86	0.75	0.71	1.01	1.02	1.02	0.87	0.79	0.79
SAA	1.48	1.52	1.71	1.74	1.92	1.47	1.46	1.46	1.63	1.73	1.81
His	1.52	1.70	1.53	1.38	1.58	1.85	1.53	1.72	1.81	1.81	1.86
Ile	1.22	1.39	1.47	1.58	1.53	1.45	1.43	1.48	1.52	1.58	1.61
Leu	1.44	1.31	1.23	1.18	1.14	1.36	1.33	1.33	1.27	1.22	1.23
Lys	1.05	1.23	1.20	1.29	1.33	1.26	1.27	1.30	1.24	1.30	1.32
Thr	1.46	1.40	1.97	2.05	2.26	1.23	1.24	1.19	1.69	1.86	1.92
Val	1.02	1.03	0.93	0.88	0.88	0.99	1.00	1.00	1.15	1.09	1.11
DIAAS (%)	94 AAA	94 AAA	86 AAA	75 AAA	71 AAA	99 Val	100 Val	100 Val	87 AAA	79 AAA	79 AAA
DIAA calculations for older children, adolescents and adults**											
AAA	1.19	1.19	1.09	0.95	0.90	1.28	1.30	1.29	1.11	1.00	1.00
SAA	1.74	1.79	2.01	2.04	2.26	1.73	1.71	1.71	1.91	2.03	2.12
His	1.90	2.13	1.92	1.73	1.98	2.32	1.91	2.15	2.27	2.26	2.32
Ile	1.30	1.49	1.57	1.69	1.64	1.54	1.53	1.58	1.62	1.68	1.71
Leu	1.55	1.41	1.33	1.27	1.23	1.48	1.44	1.44	1.37	1.32	1.33
Lys	1.25	1.47	1.42	1.53	1.58	1.49	1.51	1.55	1.47	1.54	1.56
Thr	1.82	1.74	2.44	2.54	2.81	1.53	1.53	1.48	2.09	2.31	2.38
Val	1.10	1.10	1.00	0.96	0.95	1.07	1.07	1.07	1.24	1.17	1.19
DIAAS (%)	110 Val	110 Val	100 Val	95 AAA	90 AAA	107 Val	107 Val	107 Val	111 AAA	100 AAA	100 AAA

AAA: aromatic amino acids (Phenylalanine + Tyrosine), SAA: sulfur amino acids (Methionine + Cystine), His: histidine, Ile: isoleucine, Leu: leucine, Lys: lysine, Thr: threonine, Val: valine. DIAA ratios for tryptophan (Trp) were not calculated due to the very low and inaccurate quantities caused by the acidic hydrolyzing procedure of pre-treatment.

* IAA references for young children (6 months to 3 years old) are expressed as mg amino acid/g protein and they are 52 for AAA, 27 for SAA, 20 for His, 32 for Ile, 66 for Leu, 57 for Lys, 31 for threonine, 43 for Val (FAO, 2013).

** IAA references for older children, adolescents and adults are expressed as mg amino acid/g protein and they are 41 for AAA, 23 for SAA, 16 for His, 32 for Ile, 61 for Leu, 48 for Lys, 25 for threonine, 40 for Val (FAO, 2013).

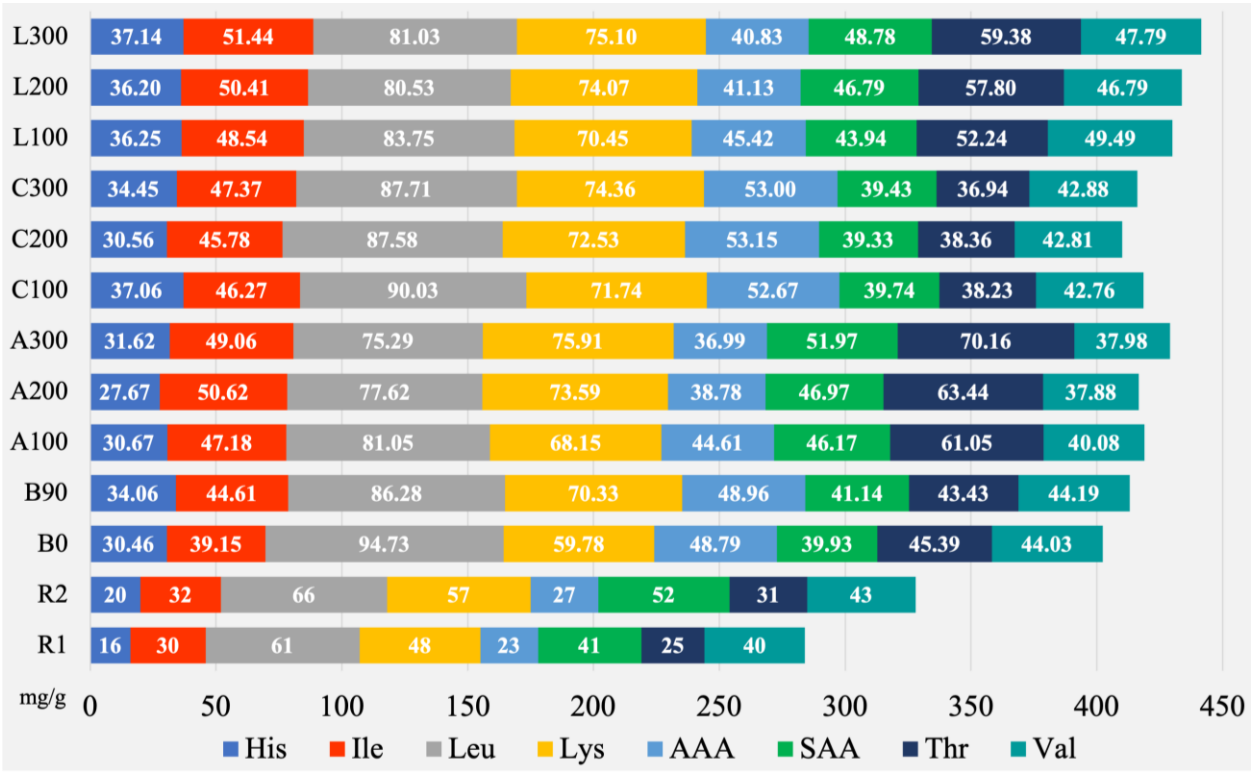

Figure 3. Comparisons of adult daily recommended indispensable amino acids (IAA) values for humans and IAA compositions (mg) per g of proteins from Black Sea salmon fillets at the end of the 90-day feeding trials. Basal diet-fed groups in the initial (0) and the last day (90) of the feeding trials were described as B0 and B90, respectively. For each carotenoid group, 100ppm, 200ppm, and 300ppm carotenoid-containing diets expressed as A100, A200, and A300 for astaxanthin, C100, C200, and C300 for canthaxanthin, L100, L200, and L300 for lycopene, respectively. Daily IAA requirement reference values, which were abbreviated as R1 for older children, adolescents, and adults and R2 for young children, were used as described by the WHO/FAO/UNU (2007). Where His: histidine, Ile: isoleucine, Leu: leucine, Lys: lysine, AAA: aromatic amino acids (phenylalanine + tyrosine), SAA: sulfur amino acids (methionine + cystine), Thr: threonine, Val: valine.

decreased statistically in all groups (P≥0.05). Even though crude fat content decreased in the lycopene group, this decrease did not adversely affect the lipid composition. The highest PUFA amount was detected in lycopene groups. The fatty acid profile of Black Sea salmon is shown in Table 4. It can be concluded that the decrease in crude fat was due to saturated fats. Some feeding studies on mice with lycopene-containing products have indicated that lycopene plays a role in weight control by activating lipid enzymes (Kim et al., 2012). Although it is too early to suggest anything explicit about the fat-burning feature of lycopene, the present study conducted on Black Sea salmon shows that the saturated fatty acids in the fish muscle decreased with lycopene intake. At the end of the trials, dihomo-γ-linolenic acid, EPA, and DHA were increased in all groups. Arachidonic acid was increased in both astaxanthin and canthaxanthin groups, while alpha-linolenic acid was increased only in astaxanthin groups (P≤0.05). These fatty acids are highly reactive, with long carbon chains and multiple double bonds highly susceptible to oxidation (Hossain 2011). We put forth those carotenoids, potent antioxidants, protecting these valuable fatty acids against free radicals by preventing muscle autooxidation.

The lipid quality indices of Black-Sea salmon fillets measured after the 90-day feeding experiment are shown in Table 5. The results indicate an increase in the PUFA/SFA ratio, fish lipid quality (FLQ), and health-promoting index (HPI) throughout the feeding trials (P≤0.05). Additionally, no meaningful statistical differences were found for the LA/ALA ratio (P≥0.05). Diets characterized by high PUFA/SFA, HPI, and FLQ indices have been linked with positive effects on cardiovascular health, attributed to reductions in lipoprotein cholesterol (LDL-C) and serum cholesterol (Chen & Liu, 2020). The highest PUFA/SFA proportions were attained in the lycopene 200 ppm, 300 ppm, and canthaxanthin 300 ppm groups, aligning with their low SFA and high PUFA contents. Similarly, FLQ was highest in these groups, paralleling the increased levels of EPA and DHA (Figure 4a). The lycopene 200 ppm and 300 ppm groups exhibited the highest HPI, characterized by the highest total MUFA and PUFA contents among all trial groups (Figure 4b). In each trial, the unsaturation index (UI) increased with rising amounts of unsaturated fatty acids (Figure 4c). Similarly, the hypo/hypercholesterolemic ratio (h/H) increased with carotenoid supplementation, while the atherogenicity index (AI) decreased (P≤0.05) (Figure 4d). Lycopene

Table 4. Fatty acid composition of Black Sea salmon fillets at the end of 90-day feeding trials based on carotenoid supplementation (%)

Fatty acids	Basal Diet		Astaxanthin			Canthaxanthin			Lycopene		
	Day 0	Day 90	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm
$C_{10:0}$	0.37±0.02^{b}	0.39±0.02^{b}	0.47±0.02^{ab}	0.48±0.04^{a}	0.47±0.01^{ab}	0.51±0.04^{a}	0.49±0.03^{a}	0.52±0.02^{a}	0.44±0.03^{ab}	0.48±0.04^{ab}	0.45±0.03^{ab}
$C_{12:0}$	0.66±0.03^{a}	0.69±0.02^{a}	0.71±0.04^{a}	0.72±0.01^{a}	0.67±0.03^{a}	0.73±0.02^{a}	0.69±0.03^{a}	0.71±0.03^{a}	0.68±0.04^{a}	0.70±0.02^{a}	0.72±0.03^{a}
$C_{14:0}$	1.93±0.03^{a}	1.89±0.02^{a}	1.68±0.03^{b}	1.64±0.02^{a}	1.71±0.03^{b}	1.76±0.05^{ab}	1.80±0.04^{ab}	1.74±0.04^{ab}	1.86±0.04^{a}	1.82±0.03^{ab}	1.85±0.05^{a}
$C_{15:0}$	5.37±0.04^{a}	5.41±0.02^{a}	5.29±0.04^{ab}	5.34±0.02^{ab}	5.37±0.03^{a}	5.32±0.03^{ab}	5.27±0.04^{ab}	5.09±0.02^{b}	5.29±0.03^{ab}	5.25±0.05^{ab}	5.19±0.04^{b}
$C_{16:0}$	11.29±0.09^{a}	11.45±0.11^{a}	11.42±0.12^{a}	11.36±0.08^{a}	11.21±0.07^{ab}	11.29±0.09^{a}	11.18±0.12^{ab}	11.23±0.10^{a}	11.11±0.08^{ab}	10.64±0.13^{b}	10.34±0.14^{b}
$C_{17:0}$	2.14±0.03^{a}	2.10±0.04^{ab}	2.13±0.04^{a}	2.09±0.03^{ab}	2.04±0.03^{b}	1.85±0.05^{c}	1.82±0.03^{cd}	1.78±0.04^{d}	1.91±0.05^{c}	1.87±0.04^{c}	1.78±0.03^{d}
$C_{18:0}$	3.87±0.07^{a}	3.75±0.06^{a}	3.62±0.06^{ab}	3.67±0.04^{ab}	3.57±0.03^{b}	3.55±0.05^{b}	3.61±0.06^{ab}	3.56±0.04^{b}	3.49±0.03^{c}	3.42±0.02^{cd}	3.37±0.05^{d}
$C_{20:0}$	1.52±0.04^{c}	1.49±0.05^{c}	1.69±0.04^{b}	1.60±0.04^{b}	1.59±0.05^{b}	1.84±0.05^{a}	1.85±0.05^{a}	1.79±0.06^{a}	1.67±0.03^{b}	1.65±0.05^{b}	1.63±0.02^{b}
$C_{21:0}$	0.85±0.02^{a}	0.88±0.03^{a}	0.86±0.03^{a}	0.90±0.05^{a}	0.81±0.05^{a}	0.87±0.04^{a}	0.89±0.05^{a}	0.79±0.06^{a}	0.89±0.03^{a}	0.85±0.03^{a}	0.84±0.05^{a}
$C_{22:0}$	1.57±0.03^{ab}	1.50±0.04^{a}	1.42±0.04^{b}	1.48±0.03^{b}	1.33±0.06^{c}	1.71±0.04^{a}	1.68±0.03^{a}	1.65±0.04^{a}	1.56±0.04^{ab}	1.45±0.04^{b}	1.47±0.04^{b}
$C_{24:0}$	0.94±0.03^{a}	0.97±0.04^{a}	0.84±0.02^{ab}	0.86±0.02^{ab}	0.87±0.03^{ab}	0.81±0.02^{b}	0.82±0.02^{b}	0.82±0.03^{b}	0.90±0.02^{a}	0.87±0.03^{ab}	0.89±0.03^{a}
$\sum_{SFA}$	30.51±0.11^{a}	30.52±0.23^{a}	30.13±0.20^{a}	30.14±0.21^{a}	29.64±0.26^{b}	30.24±0.24^{bc}	30.10±0.34^{bc}	29.68±0.27^{c}	29.80±0.36^{b}	29.00±0.21^{d}	28,53±0.22^{e}
$C_{15:1}$	0.03±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.03±0.01^{a}
$C_{16:1}$	9.64±0.36^{a}	9.80±0.31^{a}	9.81±0.35^{a}	9.83±0.38^{a}	9.89±0.29^{a}	9.81±0.25^{a}	9.65±0.36^{a}	9.69±0.41^{a}	9.81±0.40^{a}	9.79±0.32^{a}	9.75±0.31^{a}
$C_{17:1}$	1.13±0.22^{a}	1.13±0.11^{a}	1.09±0.14^{a}	1.12±0.15^{a}	1.13±0.16^{a}	1.14±0.09^{a}	1.13±0.13^{a}	1.15±0.08^{a}	1.13±0.12^{a}	1.13±0.17^{a}	1.14±0.13^{a}
$C_{18:1}$	12.44±0.41^{a}	12.31±0.35^{a}	12.32±0.36^{a}	12.32±0.17^{a}	12.40±0.34^{a}	12.24±0.29^{a}	12.29±0.28^{a}	12.34±0.32^{a}	12.61±0.24^{a}	12.80±0.31^{a}	13.11±0.49^{a}
$C_{20:1}$	1.26±0.22^{a}	1.27±0.16^{a}	1.26±0.28^{a}	1.27±0.29^{a}	1.30±0.31^{a}	1.30±0.14^{a}	1.33±0.13^{a}	1.34±0.15^{a}	1.33±0.28^{a}	1.32±0.15^{a}	1.32±0.19^{a}
$\sum_{MUFA}$	24.50±0.07^{b}	24.54±0.20^{b}	24.51±0.22^{b}	24.57±0.23^{b}	24.76±0.29^{ab}	24.53±0.15^{b}	24.43±0.19^{b}	24.55±0.26^{b}	24.92±0.25^{ab}	25.08±0.17^{a}	25.35±0.19^{a}
$C_{18:2}$	10.10±0.21^{a}	10.11±0.29^{a}	10.27±0.24^{a}	10.32±0.27^{a}	10.30±0.29^{a}	10.20±0.18^{a}	10.31±0.16^{a}	10.34±0.18^{a}	10.16±0.18^{a}	10.37±0.19^{a}	10.36±0.31^{a}
$C_{18:3}$	1.22±0.09^{a}	1.20±0.05^{b}	1.23±0.04^{a}	1.25±0.06^{a}	1.27±0.07^{a}	1.22±0.09^{a}	1.20±0.08^{a}	1.20±0.07^{a}	1.26±0.21^{a}	1.27±0.21^{a}	1.28±0.21^{a}
$C_{20:2}$	0.64±0.13^{a}	0.64±0.19^{a}	0.66±0.18^{a}	0.67±0.17^{a}	0.67±0.18^{a}	0.67±0.18^{a}	0.65±0.09^{a}	0.66±0.11^{a}	0.66±0.17^{a}	0.65±0.16^{a}	0.64±0.17^{a}
$C_{20:3}$	0.84±0.09^{b}	0.83±0.04^{b}	0.86±0.06^{b}	0.91±0.05^{a}	0.94±0.04^{a}	0.87±0.05^{ab}	0.93±0.04^{a}	0.94±0.05^{a}	0.98±0.16^{ab}	1.14±0.18^{a}	1.15±0.16^{a}
$C_{20:4}$	1.11±0.04^{b}	1.11±0.03^{b}	1.15±0.04^{a}	1.18±0.05^{a}	1.20±0.05^{a}	1.18±0.03^{a}	1.19±0.04^{a}	1.18±0.05^{a}	1.13±0.16^{a}	1.14±0.09^{a}	1.15±0.09^{a}
$C_{20:5}$	5.07±0.07^{b}	5.13±0.09^{b}	5.23±0.18^{a}	5.27±0.14^{a}	5.34±0.09^{a}	5.25±0.06^{ab}	5.33±0.04^{a}	5.39±0.05^{a}	5.29±0.06^{b}	5.36±0.11^{ab}	5.44±0.07^{a}
$C_{22:5}$	1.37±0.13^{a}	1.39±0.20^{a}	1.42±0.18^{a}	1.44±0.17^{a}	1.46±0.16^{a}	1.45±0.15^{a}	1.44±0.13^{a}	1.48±0.12^{a}	1.45±0.07^{a}	1.45±0.18^{a}	1.47±0.18^{a}
$C_{22:6}$	23.88±0.25^{b}	23.92±0.3^{b}	24.23±0.3^{ab}	24.24±0.3^{ab}	24.31±0.3^{ab}	24.25±0.19^{ab}	24.42±0.21^{a}	24.57±0.22^{a}	24.27±0.18^{ab}	24.48±0.11^{a}	24.61±0.18^{a}
$\sum_{PUFA}$	44.23±0.32^{b}	44.33±0.32^{c}	45.05±0.36^{b}	45.28±0.31^{ab}	45.49±0.30^{ab}	45.09±0.41^{b}	45.47±0.23^{ab}	45.76±0.34^{ab}	45.20±0.15^{ab}	45.86±0.13^{a}	46.10±0.18^{a}

Values expressed as mean value ± standard error and different superscripts in a line represent statistical differences, and the p-value is accepted as 0.05. $\sum_{SFA}$ total saturated fatty acids, $\sum_{MUFA}$ total monounsaturated fatty acids, $\sum_{PUFA}$ total polyunsaturated fatty acids, $C_{10:0}$ capric acid, $C_{12:0}$ lauric acid, $C_{14:0}$ myristic acid, $C_{15:0}$ pentadecylic acid, $C_{16:0}$ palmitic acid, $C_{17:0}$ margaric acid, $C_{18:0}$ stearic acid, $C_{20:0}$ arachidic acid, $C_{21:0}$ heneicosylic acid, $C_{22:0}$ behenic acid, $C_{24:0}$ lignoceric acid, $C_{15:1}$ pentadecenoic acid, $C_{16:1}$ palmitoleic acid, $C_{17:1}$ heptadecenoic acid, $C_{18:1}$ oleic acid, $C_{20:1}$ eicosenoic acid, $C_{18:2}$ linoleic acid; $C_{18:3}$ α-linolenic acid, $C_{20:2}$ eicosadienoic acid, $C_{20:3}$ dihomo-γ linolenic acid, $C_{20:4}$ arachidonic acid, $C_{20:5}$ eicosapentaenoic acid (EPA), $C_{22:5}$ docosapentaenoic acid, $C_{22:6}$ docosahexaenoic acid (DHA).

Table 5. Lipid quality indices of Black Sea salmon fillets at the end of 90-day feeding trials

Indices	Basal Diet		Astaxanthin			Canthaxanthin			Lycopene		
	Day 0	Day 90	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm
PUFA/SFA	1.45±0.03^{b}	1.45±0.03^{b}	1.50±0.02^{ab}	1.50±0.02^{ab}	1.53±0.04^{ab}	1.49±0.03^{ab}	1.51±0.02^{ab}	1.55±0.03^{a}	1.52±0.03^{ab}	1.58±0.03^{a}	1.62±0.05^{a}
LA/ALA	8.28±0.32^{a}	8.43±0.27^{a}	8.35±0.23^{a}	8.26±0.25^{a}	8.11±0.29^{a}	8.36±0.26^{a}	8.59±0.24^{a}	8.62±0.32^{a}	8.06±0.20^{a}	8.17±0.33^{a}	8.09±0.30^{a}
FLQ	29.17±0.20^{c}	29.23±0.19^{c}	29.55±0.17^{b}	29.51±0.15^{b}	29.68±0.16^{b}	29.54±0.14^{b}	29.75±0.12^{ab}	29.96±0.15^{a}	29.58±0.12^{ab}	29.86±0.17^{a}	30.06±0.21^{a}
HPI	3.49±0.07^{c}	3.50±0.06^{c}	3.69±0.05^{ab}	3.75±0.04^{ab}	3.75±0.08^{ab}	3.65±0.08^{ab}	3.67±0.05^{ab}	3.72±0.05^{ab}	3.65±0.04^{b}	3.81±0.08^{a}	3.87±0.05^{a}
UI	232.08±1.57^{d}	232.69±1.48^{d}	235.87±0.94^{c}	236.74±1.01^{bc}	237.99±0.98^{b}	236.26±1.31^{bc}	237.87±1.221^{b}	239.46±1.32^{ab}	237.12±1.28^{b}	239.84±1.09^{ab}	241.45±1.55^{a}
AI	0.29±0.02^{a}	0.29±0.02^{a}	0.27±0.02^{ab}	0.27±0.02^{ab}	0.27±0.02^{ab}	0.27±0.03^{ab}	0.27±0.02^{ab}	0.27±0.01^{ab}	0.27±0.02^{ab}	0.25±0.02^{b}	0.25±0.02^{b}
TI	0.14±0.02^{a}	0.14±0.01^{a}	0.14±0.01^{a}	0.14±0.02^{a}	0.14±0.01^{a}	0.14±0.01^{a}	0.14±0.03^{a}	0.14±0.01^{a}	0.14±0.01^{a}	0.13±0.01^{a}	0.13±0.02^{a}
h/H	4.08±0.07^{c}	4.04±0.10^{c}	4.15±0.08^{bc}	4.20±0.07^{bc}	4.26±0.08^{b}	4.16±0.09^{bc}	4.23±0.08^{b}	4.25±0.11^{b}	4.24±0.13^{b}	4.46±0.14^{ab}	4.59±0.16^{a}

Values expressed as mean value ± standard error and different superscripts in a line represent statistical differences, and the p-value is accepted as 0.05. PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, LA: linoleic acid, ALA: alpha-linolenic acid, FLQ: fish lipid quality, HPI: health promoting index, UI: unsaturation index, AI: atherogenicity index, TI: thrombogenicity index, h/H: hypo/hypercholesterolemic ratio.

groups displayed the highest UI and h/H, whereas the basal groups exhibited the lowest values. No statistical differences were found in the thrombogenicity index (TI) (P≥0.05). High AI, TI, and h/H values may suggest potential adverse effects on cardiovascular health associated with certain dietary sources (Santos-Silva et al., 2002). According to existing literature, AI and TI ranges for seafood in general are 0.21-1.07 and 0.09-0.87, respectively. Specific to Atlantic salmon (*Salmo salar*), the ranges are 0.19-0.43 for AI and 0.18-0.22 for TI. For Brown trout (*Salmo trutta*), the reported ranges are 0.64-0.72 for AI and 0.21-0.30 for TI (Chen & Liu 2020; Dal Bosco et al., 2013; Molversmyr et al., 2022). The results suggest that each carotenoid supplementation contributed to an enhancement in the lipid quality of Black Sea salmon fillets. Notably, the lycopene 300 ppm group was identified as the most valuable in terms of lipid quality. Many lipid quality indices primarily consider alterations in saturated and unsaturated fatty acid content (Chen & Liu 2020), and the improved lipid quality observed in lycopene-fed fish may be attributed to lycopene's reducing effect on saturated fatty acid content, as mentioned earlier.

Conclusion

This study evaluated the fillet quality in Black Sea salmon fed with carotenoid-supplemented diets. The findings demonstrate that all three carotenoid supplements positively contribute to the amino acids and total protein content of salmon meat. While crude fat and texture increased with astaxanthin and canthaxanthin supplementation, they exhibited a decrease in lycopene groups compared to initial values (P≤0.05). Remarkably, among the groups, the highest polyunsaturated fatty acid ratio was observed in lycopene-fed fish. The evaluation of lipid quality indices revealed noteworthy improvements with carotenoid supplementation. The PUFA/SFA ratio, FLQ, and HPI increased with feeding trials, showcasing positive effects on cardiovascular health. Lycopene supplementation, particularly in the 200 ppm and 300 ppm groups, displayed the highest PUFA/SFA ratios, FLQ, and HPI indices, underlining its potential as a valuable dietary addition. The study also evaluated the salmon fillets' digestible indispensable amino acid score (DIAAS). Notably, carotenoid supplementation, especially with canthaxanthin, significantly elevated DIAAS values in infants and toddlers (6 months–3 years), categorizing the fillets as being excellent sources of protein. In children aged >3 years, adolescents, and adults, the lycopene 100 ppm group showed increased DIAAS scores, designating it as an excellent protein source. Considering the favorable effects of astaxanthin and canthaxanthin supplementation on meat quality and the positive impact of lycopene on lipid quality indices, future investigations should explore incorporating these carotenoid additives into feed

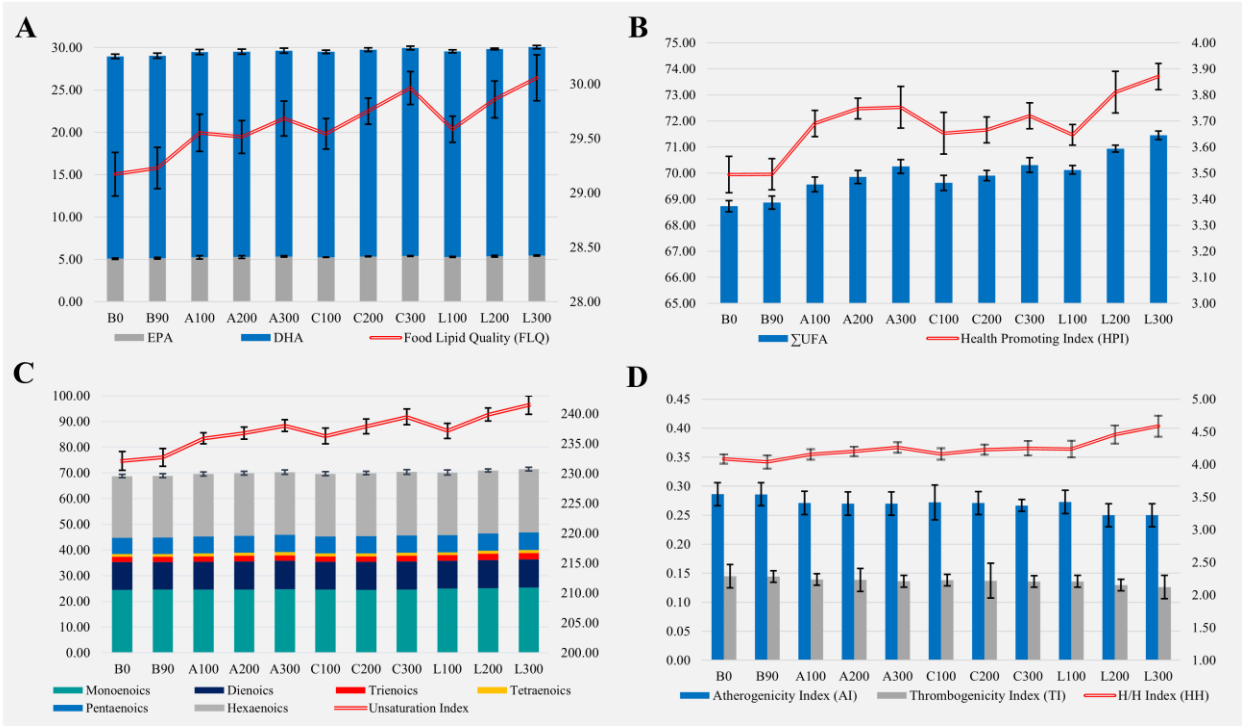

Figure 4. Changes of lipid quality indices at the end of 90-day feeding trials. Data were expressed as mean values, and standard errors were integrated into graphics. Basal diet-fed groups in the initial (0) and the last day (90) of the feeding trials were described as B0 and B90, respectively. For each carotenoid group, 100ppm, 200ppm, and 300ppm carotenoid-containing diets expressed as A100, A200, and A300 for astaxanthin, C100, C200, and C300 for canthaxanthin, L100, L200, and L300 for lycopene, respectively. A: The increase of food lipid quality (FLQ) with EPA and DHA, B: The increase of health-promoting index (HPI) parallel with total unsaturated fatty acids (UFA), C: Increase of unsaturation index (UI) and unsaturated fatty acid groups, D: Changes of atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic/hypercholesterolemic ratio (HH).

rations in specific combinations. Examining the impact of different doses of these carotenoid mixtures on both meat quality, growth performance, and amino acid profiles is a subject that warrants further research. Lycopene, recognized as a potent antioxidant actively involved in various metabolic reactions, stands out as a significant bioactive substance extensively studied in the medical community. However, its application in aquaculture remains relatively limited. Further studies in this domain will provide valuable insights into lycopene's role in fish metabolism and contribute to expanding its application in aquaculture practices. This research, with its comprehensive evaluation of meat quality, lipid quality indices, and amino acid profiles, lays the foundation for future investigations to deepen our understanding of the intricate relationships between carotenoid supplementation, meat quality, and the overall health and growth performance of Black Sea salmon.

Ethical Statement

All feeding trials and sampling studies were performed according to ethical rules. The study was ethically approved with the ETIK-2017/1 code by the Ethical Committee of Animal Experiments of Central Fisheries Research Institute, Ministry of Agriculture and Forestry, Republic of Türkiye.

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Author Contribution

ECÇ: Conceptualization, Methodology, Investigation, Data curation, Validation, Writing - Original draft. NB: Conceptualization, Writing - Review & editing, Supervision.

Conflict of Interest

There is no conflict of interest between the authors.

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