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Original Article
Özgün Araştırma

Investigation of the effect of astaxanthin administration on the amount of sialic acid in muscle tissue in Rainbow trout

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Abstract: Astaxanthin is an oxidized carotenoid component responsible for pigmentation, protecting essential polyunsaturated fatty acids from oxidation, immune response, communication, reproductive behavior, and improved reproduction in aquatic animals. In aquaculture, astaxanthin is a pigment used to give rainbow trout flesh its distinctive pinkish tint. It impacts cell recognition, communication, signaling, cellular aggregation and development, infections, tumor growth and metastasis, immunology, reproductive biology, and neurology. The purpose of this study was to look into the effects of astaxanthin supplementation on levels of sialic acid, which is a substance related to cell integrity, in rainbow trout muscle tissue. Forty female rainbow trout were used in the study. Fish were divided into two groups. The experimental group (n=20) received a daily dose of astaxanthin of 50 ppm/day for 225 days, whereas the control group (n=20) received a standard fish meal. The concentration of sialic acid in fish muscle tissue was evaluated using the enzyme-linked immunosorbent assay (ELISA) method. The sialic acid level in muscle tissue of fish fed a standard fish ration was 111.97 ± 4.56 µg/g, while the sialic acid level in muscle tissue of fish fed astaxanthin was 110.33 ± 8.54 µg/g. In our research, we found that adding astaxanthin to the fish diet had no effect on the amount of sialic acid in the fish, which is crucial in human nutrition. As a result, it was revealed that dietary astaxanthin did not influence the level of sialic acid in fish muscle tissue ($p > 0.05$).

Keywords: Astaxanthin; Rainbow trout; Sialic acid

Gökkuşağı alabalığı rasyonuna eklenen astaksantinin kas dokusu siyalik asit miktarı üzerine etkisinin araştırılması*

Özet: Astaksantin, sucul hayvanlarda, pigmentasyondan, temel çoklu doymamış yağ asitlerinin oksidasyondan korunmasından, bağışıklık tepkisinden, iletişimden ve üremeden sorumlu oksitlenmiş bir karotenoid bileşeni olup gökkuşağı alabalığına, etinin kendine özgü pembemsi rengini vermek için kullanılmaktadır. Sialik asit hücrelerin tanınmasında, iletişiminde, sinyalleşmesinde, kümelenmesinde ve gelişmesinde, enfeksiyonlarda, tümör büyümesinde ve metastazında, immunolojide, üreme biyolojisinde ve nörolojide önemli etkilere sahiptir. Bu çalışmanın amacı, astaksantin takviyesinin, gökkuşağı alabalığı kas dokusunda hücre bütünlüğü ile ilgili olan siyalik asit seviyeleri üzerine etkilerini araştırmaktır. Sunulan çalışmada 40 adet dişi Gökkuşağı alabalığı kullanıldı. Balıklar iki gruba ayrılmış olup deney grubuna (n=20) 225 gün boyunca günlük 50 ppm/gün astaksantin verilirken, kontrol grubu (n=20) standart balık rasyonu ile beslendi. Balık kas dokusunda siyalik asit konnsantrasyonu enzim bağlı immünosorbent analiz (ELISA) yöntemi ile belirlendi. Normal balık rasyonu ile beslenmiş balıkların kas dokusu siyalik asit düzeyi 111,97 ± 4,56 µg/g ve rasyonuna astaksantin eklenmiş balık kas dokusunda siyalik asit düzeyi 110,33 ± 8,54 µg/g olarak bulundu. Araştırmamızda, balık diyetine astaksantin eklenmesinin, insan beslenmesinde çok önemli olan balıklardaki siyalik asit miktarında değişikliğe neden olmadığı belirlendi. Sonuç olarak rasyona eklenen astaksantinin balık kas dokusu siyalik asit miktarı üzerine bir etkisi olmadığı ortaya konuldu.

Anahtar kelimeler: Astaksantin; Gökkuşağı alabalığı; Siyalik asit

Introduction

Different feeding techniques are employed in industrial aquaculture to promote consumer preference of fish. The consumer's choice of fish is frequently influenced by the pink color of the fillet, therefore farmed salmonids are frequently fed diets enhanced with carotenoids (Elia et al., 2019). The common red to pink flesh color of farmed trout is developed by using xanthophylls such as astaxanthin and cantaxanthin (Rehulka, 2000; Elia et al., 2019). Astaxanthin is an oxidized carotenoid component of the xanthophyll family that gives wild salmonids their characteristic red to pink tint (Schiedt et al., 1986; Storebakken and No, 1992). In aquatic species, the primary roles of astaxanthin include pigmentation, protection of vital polyunsaturated fatty acids from

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oxidation, immunological response, communication, reproductive behavior, and reproduction improvement (Lorenz and Cysewski, 2000; Guerin et al., 2003). *Haematococcus pluvialis*, unicellular green algae, is a significant generator of the carotenoid astaxanthin (Harker and Young, 1995). Astaxanthin protects cells from oxidative damage by neutralizing singlet oxygen, scavenging radicals to avoid chain reactions, maintaining membrane structure by preventing lipid peroxidation, boosting immune system function, and modulating gene expression (Fakhri et al., 2018). Its potent antioxidant qualities, such as minimizing oxidative damage caused by free radicals in the human body and preventing the oxidation of unsaturated fatty acids, give therapeutic effects in various disorders (Fang et al., 2019).

Sialic acid is a nine-carbon alpha-keto acid monosaccharide, an acylated derivative of neuraminic acid, and a terminal component of the oligosaccharide chains of numerous glycoproteins and glycolipids (Schauer et al., 1995; Angata and Varki, 2002). It can be found in the glycan chains of glycolipids or glycoproteins, on the cell surface as a glycoconjugate, or as a terminal monosaccharide in carbohydrate chains such as oligosaccharides and polysaccharides (Bork et al., 2005; Schauer and Kamerling, 2018; Pongracz et al., 2019). Glycoproteins on the surface of cells are crucial for maintaining both the cells composition and function (Fukuda, 1996). Sialic acids have more natural modifications than monosaccharides with five or six carbons, such as aldose and ketose (Deng et al., 2013). Sialic acid's high structural complexity and various characteristics contribute to various biological activities in human nutrition (Angata and Varki, 2002; Chen and Varki, 2010). Neuraminic acid is a sialic acid precursor not found in free form. Neu5Ac is mammals' most prevalent N-acetylneuraminic acid (C11H19NO9) (Deng et al., 2013). Sialic acid can be O-acetylated, O-methylated, O-sulphated, or phosphorylated to generate distinct isoforms. In contrast, neuraminic acid can be N-acetylated to form Neu5Ac and N-glycosylated to form Neu5Gc, all of which play critical roles in the mammalian physiological system (Huang et al., 2015).

Sialic acids are commonly found in higher animals. However, it is unclear whether the sialic acids are created by the animals themselves, come from their food, or are created by bacteria. Sialic acid concentrations have been detected in subcellular membrane fractions of different cell types, with plasma membrane having the highest values and smooth endoplasmic reticulum showing significantly lower concentrations (Schauer and Kamerling, 2018). High amounts of sialic acids can be found in glycoproteins, glycolipids like gangliosides, or polysaccharides. These molecules are typically found on the outer cell membrane, where they are joined to other sugars like galactose or N-acetyl galactosamine via glycosidic bonds (Narayanan, 1994). Sialic acid units play an important role in cellular recognition, communication, signaling, cellular aggregation, and development, managing the lifespan of glycoconjugates in animals, bacterial and viral infections, tumor growth and metastasis, immunology, microbiome biology, reproductive biology, and play a significant part in neuroscience. They are the terminal element of carbohydrate chains like glycoproteins and glycolipids, oligosaccharides, capsular and tissue polysialic acids, bacterial lipooligosaccharides, and lipopolysaccharides can exist in a variety of chemical forms that alter their biology significantly. O-acetyl and N-glycolyl groups effectively display the biological functions of sialic acids. Many genes and enzymes regulate the anabolism and catabolism of sialic acids, which play a role in pathological and normal cellular activities (Schauer, 2009; Chen and Varki, 2010). N-acetylneuraminic acid (NeuAc), the most prevalent sialic acid, is a powerful acid due to the carboxylate close to the anomeric carbon in its structure. The exocyclic glycerol side chain (C-7, C-8, C-9) allows for hydrogen bonding. The N-acetyl group allows hydrophobic interactions. Each of these fragments contributes significantly to the binding characteristics and functions of sialoglycans (Roth et al., 1992; Malykh et al., 1999; Chen and Varki, 2010; Repnikova et al., 2010; Di et al., 2017). Sialic acids in the animal organism are typically based on neuraminic acid (Neu), which exists in N-acetyl or N-glycolyl forms or on 2-keto-3-deoxy-nulosonic acid (Schnaar et al., 2014).

According to researchs, astaxanthin reduces oxidative stress. However, there isn't much information about how it affects aquatic species' cell membrane integrity. In this study, it was aimed to investigate potential changes on sialic acid levels, which is related to cell integrity, in rainbow trout muscle tissue after supplementation with 50 ppm astaxanthin.

Materials and Methods

Animal design

The forty female rainbow trout utilized in the study were raised commercially in marine cages, have an average weight of 2600-2750 g, and are 24-26 mont-

hs old. The fish were separated into two groups: experimental and control. The fish were fed with commercial Biomar sagun 792 A50 feed. The experimental group (n=20) received an average dose of astaxanthin of 50 ppm/day for 225 days (Song, et al., 2017) whereas the control group (n=20) received a standard fish ration. Fish were fed *ad libitum* and harvested from March to June when the sea water's temperature was between 8-18 C. For this study, an application to the ethics committee was made. Since it was not an experimental search, the committee ruled that approval was not required for this study.

Sialic acid measurement

Enzyme-linked immunosorbent assay (ELISA) assay kit (MBS8309610, MyBioSource, Inc., San Diego, CA, USA) was used to measure sialic acid concentration in fish muscle tissue samples. The competitive inhibition enzyme immunoassay method is used in this assay. Absorbance was measured at 450 nm with a microplate reader (Infinite F50, Tecan Austria GmbH, Grödig, Austria).The ELISA stages were carried out exactly as the manufacturer instructed. The kit's measurement range was 1 mg/dL to 300 mg/dL, with a minimum detectable level of 0.47 mg/dL. Results were calculated and presented in µg/g of muscle tissue.

Statistical analysis

The SPSS 22.0 package program was used for statistical analysis of study findings. Before the significance test, the Shapiro-Wilk test was performed to check for normality, which is one of the parametric test assumptions. The results of repeated experiments were expressed as mean ± standard deviation (SD). Independent samples T-test was used to reveal the statistical significance between groups. Results with a p-value of 0.05 or below were regarded as significant. Results with a p-value of 0.05 or below were regarded as significant.

Results

There was no statistically significant difference in muscle tissue sialic acid levels between the control and astaxanthin groups ($p>0.05$). The sialic acid levels in the muscle tissue of the control and astaxanthin groups were recorded as 111.97 ± 4.56 µg/g and 110.33 ± 8.54 µg/g, respectively. Postmortem examination of the fish revealed no macroscopic findings other than the desired pinking of the flesh.

Table 1. Sialic acid levels (µg/g) of the groups (Mean ± SD), $p>0.05$, n= 40.

	Control	Astaxanthin
Sialic acid levels	111.97 ± 4.56	110.33 ± 8.54

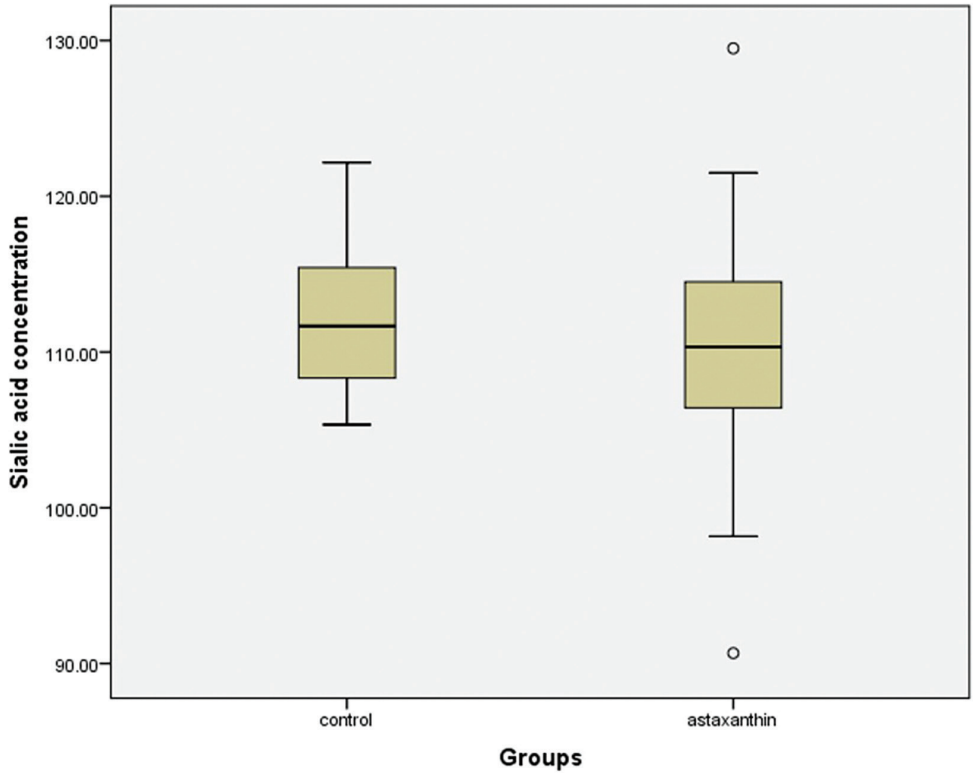

Figure 1. Sialic acid concentrations (µg/g) of the groups. $p>0.05$, Independent samples T-test

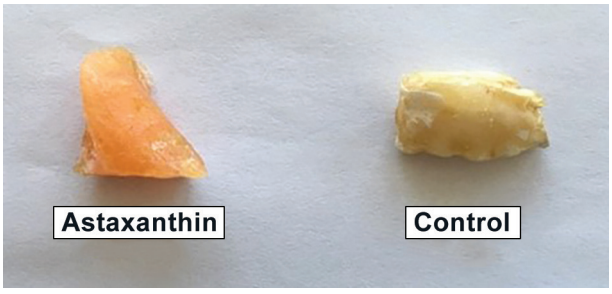

Figure 2. Fish muscle color variations of the groups

Discussion

Sialic acid has been postuated important molecule in human stres biology due to its role in neuronal development and its antiviral, anti-hypertensive, and antitumor potentials (Guo et al., 2006; Zhou et al., 2017; Abdul Khalid et al., 2019; Wang et al., 2019). Sialic acid contributes to the development and healthy functioning of the vertebrate nervous system (Schnaar et al., 2014). Sialic acid extracted from edible bird's nests has been shown to improve cognitive performance in mice (Abdul Khalid et al., 2019). Scientific research has shown that sialic acid significantly impacts the immune system. Sialic acid has been shown to have antiviral properties against the influenza virus and boost human immunity (Guo et al., 2006). Through neuraminidase inhibition, Neu5Ac has been found to have an antiviral effect on the influenza A virus by directly inhibiting viral nucleic acid and viral non-structural protein type 1 genes (Haghani et al., 2016). Neu5Ac, a type of sialic acid, has been shown to suppress influenza A viruses as effectively as commercial antiviral medicines (Haghani et al., 2017). Sialic acid-containing components act as anti-adhesive agents against the pathogenesis of *Helicobacter pylori* and play a role in the prevention and treatment of the disease has been revealed (Sun et al., 2020). In a vascular tension test on the mesenteric artery of rats, it has been reported that after the injection of Neu5Ac, a dose-dependent significant blood vessel dilation occurred, and sialic acid could have a potential anti-hypertensive effect (Wang et al., 2019). Hydrophilic and negatively charged sialic acid has been determined that supports the stabilization of red blood cells by preventing blood component aggregation (Ghosh, 2020). Sialic acids act as potent immune modulators on the surface of cancer cells has been reported (Bull et al., 2015). Yadav et al. (2020), suggested that comparing controls to Alzheimer's disease patients, the level of free sialic acid in plasma has increased along with the parameters associated with oxidative stress, and this molecule possesses antioxidant properties to protect against the greater level of oxidative stress.

In addition to synthesizing sialic acid in the human body, animal-derived sialic acid plays a significant role in human nutrition. Knowing the dietary sources of sialic acid and its level is crucial when choosing foods. Adequate and balanced nutrition plays a vital role in protecting and maintaining the health of humans and animals and contributes to improving the quality of life. Fish, which contains growth and development factors and protective molecules, has an essential place among the nutrients the organism needs in mammals' postnatal development (Crawford and Broadhurst, 2012; Yılmaz et al., 2018; Sarojnalini and Hei, 2019). It is essential to know the micronutrient levels in fish tissues, which have an important value in human nutrition (Balami et al., 2019; Mishra, 2020).

Higher animals frequently contain sialic acids. However, it is unknown whether the sialic acids are produced by bacteria, come from the food they eat, or are secreted by the organisms themselves (Schauer and Kamerling, 2018). According to our research, astaxanthin is not a source of sialic acid in fish muscle tissue, and it also didn't derived from oral administration of astaxanthin. Sialic acid is typically found in carbohydrate antigens related with tumors, including those that have been clinically recognized as tumor indicators. Accordingly, the malignant and metastatic phenotypes of several forms of cancer have been linked to the overexpression of this compound on cell membranes. Sialic acid is a crucial molecular target for both diagnostic and therapeutic strategies (Matsumoto et al., 2010). Our results demonstrated that astaxanthin had no impact on the integrity of cellular membranes, apoptotic metabolism, or oncogenic constellation in fish.

In our study, it was found that oral astaxanthin administration had no statistical effect on sialic acid concentration in fish muscle tissue. On the other hand, in a study using *Capoeta capoeta* exposed to glyphosate pollution in the aquatic environment, plasma Total Sialic Acid (TSA) and malondialdehit (MDA) levels related to lipid peroxidation were dramatically affected (Kaya et al., 2012). Sialic acid is well-known for its utility in diagnosing and prognosis of inflammatory disorders. Sialic acid levels were higher in the infected group than in the non-infected group. During various disorders, sialic acid concentrations have been defined as high. Total sialic acid and MDA levels were higher in infected animals with Saprolegniasis than in the healthy group. Beca-

use acute-phase proteins, such as 1-acid glycoprotein, are sialated glycoproteins, an increase in TSA in these conditions may be attributed to the rise in acute phase proteins (Azimzadeh and Amniattalab, 2017). The immunized rainbow trout groups had significantly higher Total Sialic Acid levels in the kidney, spleen, and liver than the controls (Heidarieh et al., 2019). The accumulation of Cu and Cd in the tissues of *Cyprinus carpio* has been observed to have various effects on the sialic acid concentration of the tissues. Cu, a beneficial ion for proper tissue function, has an antagonistic impact on Cd-induced changes in sialic acid *in vivo*. On the other hand, Cd has no physiological role and has a synergistic impact with sialic acid. It is also possible that these findings result from direct metal ion impacts on sialic acid metabolism (Aktaç et al., 2010).

Conclusion

Sialic acid, found on the surface of animal cells, plays an important vital cellular processes such as cell-cell communication, cell signaling, and immunological responses. Sialic acid, a component of gangliosides that aids in the development and good functioning of the vertebrate nervous system, plays a crucial part in transmitting and storing information in the brain. The considerable structural complexity and various properties of sialic acid contribute to various biological activities in human nutrition. Animal-derived sialic acid serves an essential function in human nutrition. An adequate and balanced diet is critical for safeguarding and sustaining human and animal health and boosting overall quality of life. Fish, which contains growth and development components and protective compounds, plays a significant role in the postnatal development of mammals as a source of nutrients. It's crucial to understand the amounts of micronutrients in fish tissues, as they play a significant role in human nutrition. Astaxanthin protects cells from oxidative damage while maintaining membrane structure, enhancing immune system function, and altering gene expression. This research examined the possible changes in Rainbow trout muscle tissue sialic acid levels after supplementation with 50 ppm astaxanthin, which is connected to cell integrity. According to our findings, oral astaxanthin administration did not significantly influence sialic acid levels in fish muscle tissue.

Animal Rights Statement: This study was conducted under the supervision of the Ondokuz Mayıs University. In addition, permission (Accept number: 2018/22 30/03/2018) was obtained from the ethics committee of experimental animals of Ondokuz Mayıs Üniversity.

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