Anatomical and histological investigation of the effect of superoxide dismutase on eye muscles in Tuj sheep

Gülseren KIRBAŞ DOĞAN[1,a,✉], Sevda ELİŞ YILDIZ[2,a], Ebru KARADAĞ SARI[1,b], Mükremin ÖLMEZ[1,c,] Tarkan ŞAHİN[1,c]

1aKafkas University, Veterinary Faculty, Department of Anatomy, Kars, Turkey

2a Kafkas University, Health Sciences Faculty, Department of Midwifery, Kars, Turkey

1b Kafkas University, Veterinary Faculty, Department Histology and Embryology, Kars Turkey

1c Kafkas University, Department of Animal Nutrition and Nutritional Diseases, Kars, Turkey

[1a]ORCID: 0000-0003-3770-9956; [2a]ORCID: 0000-0002-3585-6648; [1b]ORCID: 0000-0001-7581-6109; [1c]ORCID: 0000-0002-5003-3383; [1c]ORCID: 0000-0003-0155-2707.

[✉]Corresponding author: glsrn36@gmail.com

Abstract

In the study, 16 eye muscles belonging to 8 male Tuj breed sheep, aged around 2-3 months, were used. The research was carried out on two groups: control and study groups. The mean and standard deviation values of all measurements obtained as a result of anatomical examinations and the differences between the control and study groups were determined by the 'Independent Samples T' test in the SPSS package program While no additive was added to the control group diet, an antioxidant feed additive rich in superoxide dismutase was added to the study group diet. As a result of anatomical evaluation, the longest muscle in the control group was musculus obliquus dorsalis (34.43 mm) and the shortest muscle was musculus retractor bulbi (21.74 mm). In the study group, the longest muscle was musculus rectus dorsalis (34.5 mm) and the shortest

muscle was musculus retracor bulbi (22.27 mm). Mallory's modified triple staining (Triple) was applied for histolohical examination. In our study, it was observed that the musculus rectus dorsalis, musculus rectus ventralis, musculus rectus medialis, musculus rectus lateralis, musculus obliquus dorsalis, musculus obliquus ventralis and musculus retractor bulbi muscles obtained from Tuj sheep in the study and control groups consisted of a striated skeletal muscle system and showed transverse striations. What was clearly seen in both anatomical and histological evaluation was that superoxide dismutase had a positive effect on the musculus rectus medialis, one of the eye muscles. It is thought that the presented study will contribute to intraocular eye surgery operations and research relating eye diseases to nutrition.

Keywords: *anatomy; eye muscle; superoxide dismutase; Tuj sheep*

1. Introduction

The homeland of Tuj sheep is Turkey. It is grown in Kars, Ardahan, and Iğdır provinces. The combination is efficient. The yield direction is combined, meat, wool, and milk. Its body is generally small and bright white in color. There is black pigment on the skin, around the eyes and mouth, and on the feet (1). Antioxidant systems are potentially important for eye tissues. Oxygen free radicals and antioxidant systems are thought to play a role in pathological processes in the eye, including cataracts. It has been observed that superoxide dismutase has a protective effect as well as its therapeutic properties (2).

There are four rectus muscles that move the eye: dorsal, ventral, lateral, and medial. These muscles are responsible for turning the eyeball up and down and in and out. Musculus obliquus dorsalis turns the eyeball downwards and outwards. Musculus obliquus ventralis is responsible for turning the eyeball up and out. Musculus retractor bulbi pulls the eyeball into the orbit (3, 4).

There are studies on eye muscles in different animal species (5, 6, 7, 8). However, it has been observed that studies on eye muscles are limited on a species basis. In this sense, this study was designed to eliminate the deficiency and to evaluate the effect of superoxide dismutase on the eye muscles anatomically and histologically.

2. Material and method

2.1. Animals and management

After a one-week adaptation period, the animals were divided into two experimental groups, with 4 animals in each group. The nutrition-related part of the study was completed in 60 days. During the fattening period, the sheep were given 700 g of concentrated feed, 225 g of fresh sugar beet pulp and 200 g of wheat straw for two meals a day (08.00-16.00). Water was offered ad-libitum. While no additive was added to the control group diet, a new generation antioxidant feed additive rich with new superoxide dismutase was added to the study group diet at the level of 30g/ton. The feed additive product was supplied from a private commercial company (MeloFeed®, Lallemand Animal Nutrition, Canada).

2.2. Anatomical procedures

In the study, 16 eye muscles belonging to total 8 male Tuj breed sheep were used for around 2-3 months. Animals that reached the desired maturity were brought to the Anatomy department laboratory after slaughter. After the skin was dissected, the orbit was reached very carefully from the medial and lateral parts of the eyes. The eye and the auxiliary organs around it were removed from the eye ball. Eye muscles (mrd: musculus rectus dorsalis, mod: musculus obliquus dorsalis, mrv: musculus rectus ventralis, mrl: musculus rectus lateralis, mov: musculus obliquus ventralis, mrb: musculus retractor bulbi, mrm: musculus rectus medialis) were carefully dissected. named. The length, width and thickness of each eye muscle were measured with the help of a digital caliper. For the use of scientific terms N.A.V. (9) was applied.

2.3. Histological procedures

Tissue samples were fixed in 10% formaldehyde solution for 24 hours, then underwent routine histological procedures and were blocked in paraffin. Mallory's modified triple staining (Triple) was applied to 5 µm sections taken from these blocks to show the general structure of the tissue. The prepared preparations were examined under a light microscope (Olympus CX23, Tokyo, JAPAN). Image-j (vI. 50i) software program was used for muscle thickness measurements in the eye tissue of all groups. Muscle thickness measurements were made from a total of 40 areas in 4 different sections in each group (10).

2.4. Statistical analysis

The mean and standard deviation values of all measurements obtained as a result of anatomical examinations and the differences between the control and study groups were determined by the 'Independent Samples T' test in the SPSS package program (20.0 version, IBM Corp., Armonk, NY, US). Statistical significance level was accepted as $p<0.05$.

3. RESULTS

3.1. Anatomical results

It was determined that the nerve opticus showed a course towards the musculus retractor bulbi. The musculus retractor bulbi was between the musculus rectus ventralis and the musculus rectus lateralis. Musculus rectus dorsalis and musculus obliquus dorsalis started from the same place as a common root (Figure 1).

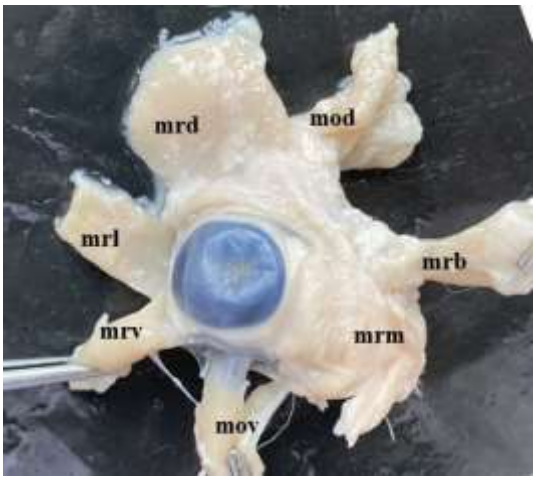


Figure 1. Eye muscles in Tuj sheep. **mrd:** musculus rectus dorsalis, **mod:** musculus obliquus dorsalis, **mrv:** musculus rectus ventralis, **mrl:** musculus rectus lateralis, **mov:** musculus obliquus ventralis, **mrb:** musculus retractor bulbi, **mrm:** musculus rectus medialis.

In the control group, the longest muscle was musculus obliquus dorsalis (34.43 mm) and the shortest muscle was musculus retractor bulbi (21.74 mm). In the study group, the longest muscle was musculus rectus dorsalis (34.5 mm) and the shortest muscle was musculus retracor bulbi (22.27 mm). In terms of direction, the longest muscle on the right side was musculus obliquus dorsalis (34.43 mm), and the longest muscle on the left side was musculus rectus dorsalis (34.5 mm).

In the control group, the widest muscle was measured as musculus rectus lateralis (11.95 mm), and the shortest muscle was measured as musculus obliquus dorsalis (8.14 mm). In the study group, the widest muscle was musculus rectus dorsalis (22.62 mm), and the muscle with the shortest width was musculus obliquus dorsalis (5.26 mm). In the control group, the muscle with the highest muscle thickness was determined as musculus rectus ventralis and the muscle with the least thickness was determined as musculus rectus dorsalis. In the study group, the muscle with the most thickness was musculus rectus medialis and the muscle with the least thickness was musculus rectus lateralis. Statistical data on eye muscles in the control and study groups

٧٦ are presented in Table 1. The evaluation of the same animals in terms of direction is shown in

٧٧ Table 2.

٧٨ **Table 1.** Some parameters of Tuj sheep eye muscles in groups

Eye muscle	Control (n=8)	Study (n=8)	P value
MRDU	25,40 ± 3,91	34,50 ± 2,70	<0,001
MRDG	11,81 ± 3,74	22,62 ± 11,75	0,037
MRDK	1,74 ± 0,52	1,74 ± 0,47	0,992
MRVU	26,62 ± 2,82	29,18 ± 3,44	0,126
MRVG	9,45 ± 0,67	8,65 ± 1,12	<0,001
MRVK	2,62 ± 0,34	1,91 ± 0,49	0,005
MRMU	25,81 ± 1,55	28,54 ± 2,58	0,022
MRMG	10,58 ± 0,66	7,63 ± 1,06	<0,001
MRMK	2,27 ± 0,34	2,03 ± 0,21	0,114
MRLU	27,42 ± 5,00	29,70 ± 1,71	0,244
MRLG	11,95 ± 0,82	9,89 ± 0,57	<0,001
MRLK	2,14 ± 0,70	1,66 ± 0,51	0,141
MRBU	21,74 ± 2,62	22,27 ± 2,78	0,699
MRBG	8,66 ± 1,06	9,33 ± 1,43	0,306
MRBK	2,13 ± 0,32	1,91 ± 0,04	0,097
MODU	34,43 ± 6,13	28,43 ± 16,65	0,355
MODG	8,14 ± 2,11	5,26 ± 1,31	0,005

MODK	2,11± 0,25	1,69 ± 0,30	0,008
MOVU	34,23 ± 1,23	27,03 ± 28,21	0,354
MOVG	7,04 ± 1,09	6,08 ± 1,21	0,005
MOVK	2,05± 1,23	1,70 ± 0,32	0,008

MRDU: Musculus rectus dorsalis length, **MRDG:** Musculus rectus dorsalis width, **MRDK:** Musculus rectus dorsalis thickness, **MRVU:** Musculus rectus ventralis length, **MRVG:** Musculus rectus ventralis width, **MRVK:** Musculus rectus ventralis thickness, **MRMU:** Musculus rectus medius length, **MRMG:** Musculus rectus medius width, **MRMK:** Musculus rectus medius thickness, **MRLU:** Musculus rectus lateralis length, **MRLG:** Musculus rectus lateralis width, **MRLK:** Musculus rectus lateralis thickness, **MRBU:** Musculus retractor bulbi length, **MRBG:** Musculus retractor bulbi width, **MRBK:** Musculus retractor bulbi thickness, **MODU:** Musculus obliquus dorsalis length, **MODG:** Musculus obliquus dorsalis width, **MODK:** Musculus obliquus dorsalis thickness, **MOVU:** Musculus obliquus ventralis length, **MOVG:** Musculus obliquus ventralis width, **MOVK:** Musculus obliquus ventralis thickness

Table 2. Directional results of some parameters of Tuj sheep eye muscles in groups

Eye muscle	**Right (n=8)**	**Left (n=8)**	**P value**
MRDU	25,40 ± 3,91	34,50 ± 2,70	<0,001
MRDG	11,81 ± 3,74	22,62 ± 11,75	0,037
MRDK	1,74 ± 0,52	1,74 ± 0,47	0,992
MRVU	26,62 ± 2,82	29,18 ± 3,44	0,126
MRVG	9,45 ± 0,67	8,64 ± 1,12	0,108
MRVK	2,62 ± 0,34	1,90 ± 0,49	0,005

MRMU	25,81 ± 1,55	28,54 ± 2,58	0,022
MRMG	10,58 ± 0,66	7,63 ± 1,06	<0,001
MRMK	2,28 ± 0,34	2,03 ± 0,21	0,118
MRLU	27,42 ± 5,00	29,70 ± 1,71	0,244
MRLG	11,95 ± 0,82	9,89 ± 0,57	<0,001
MRLK	2,14 ± 0,70	1,66 ± 0,51	0,141
MRBU	21,74 ± 2,62	22,27 ± 2,78	0,699
MRBG	8,66 ± 1,06	9,33 ± 1,43	0,308
MRBK	2,13 ± 0,32	1,91 ± 0,04	0,078
MODU	34,43 ± 6,13	28,44 ± 16,65	0,365
MODG	8,14 ± 2,11	5,26 ± 1,31	0,005
MODK	2,11 ± 0,25	1,69 ± 0,30	0,008
MOVU	33,96 ± 4,23	29,14 ± 15,44	0,365
MOVG	7,44 ± 2,10	5,29 ± 0,33	0,005
MOVK	1,98 ± 0,32	1,71 ± 0,32	0,008

MRDU: Musculus rectus dorsalis length, **MRDG:** Musculus rectus dorsalis width, **MRDK:** Musculus rectus dorsalis thickness, **MRVU:** Musculus rectus ventralis length, **MRVG:** Musculus rectus ventralis width, **MRVK:** Musculus rectus ventralis thickness, **MRMU:** Musculus rectus medius length, **MRMG:** Musculus rectus medius width, **MRMK:** Musculus rectus medius thickness, **MRLU:** Musculus rectus lateralis length, **MRLG:** Musculus rectus lateralis width, **MRLK:** Musculus rectus lateralis thickness, **MRBU:** Musculus retractor bulbi length, **MRBG:** Musculus retractor bulbi width, **MRBK:** Musculus retractor bulbi thickness, **MODU:** Musculus obliquus dorsalis length, **MODG:** Musculus obliquus dorsalis width, **MODK:** Musculus obliquus dorsalis thickness, **MOVU:** Musculus obliquus ventralis length, **MOVG:** Musculus obliquus ventralis width, **MOVK:** Musculus obliquus ventralis thickness

3.2. Histological results

In Tuj sheep in the study and control groups, musculus rectus dorsalis, musculus rectus ventralis, musculus rectus medialis, musculus rectus lateralis, musculus obliquus dorsalis, and musculus retractor bulbi had normal histological structure. When the interstitial connective tissue of the muscles in Tuj sheep in the study and control groups was examined, no change was observed in both the connective tissue cells and the distribution of connective tissue fibers. It was observed that the muscle fibers were surrounded by connective tissue containing blood vessels and nerves. In cross-sectional histological images, it was observed that the striated skeletal muscle system consisted of muscle fibers that were surrounded by the endomysium and formed muscle bundles. These bundles were seen to be arranged in groups by the epimysium. It was determined that there were transverse lines and A and I bands in the longitudinal sections (Figure 2).

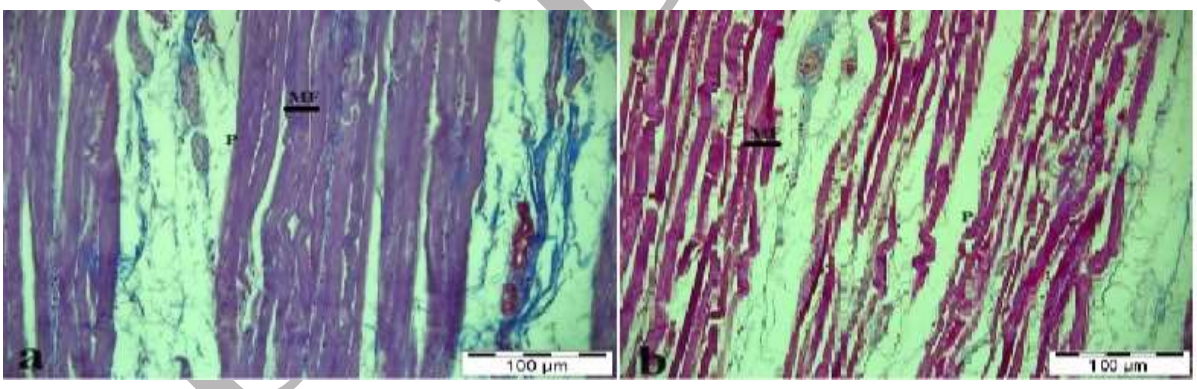
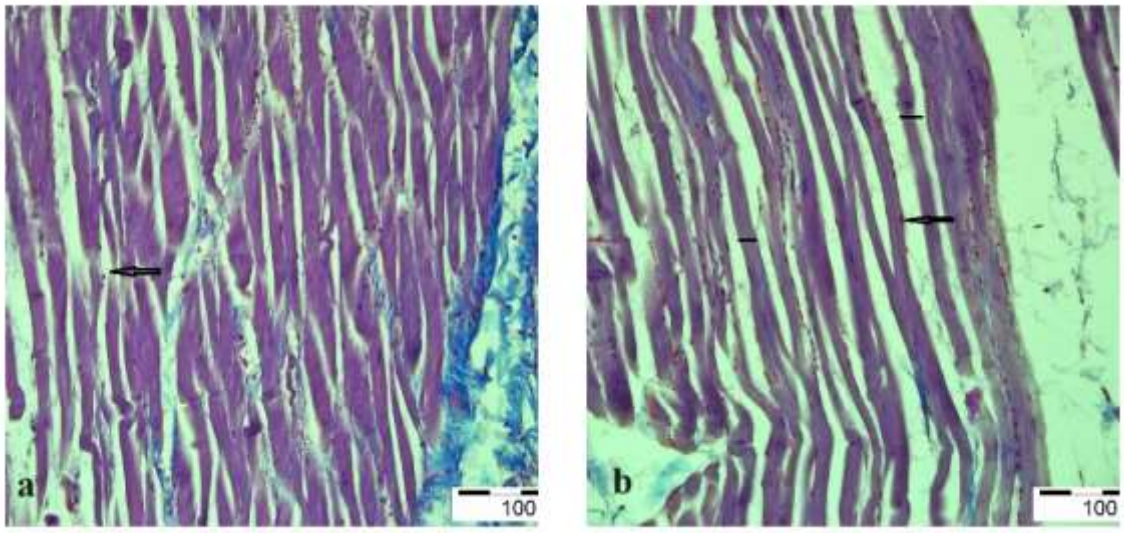


Figure 2. Tuj sheep eye muscles **a)** Study group, musculus rectus dorsalis eye muscle (right eye), **b)** Control group, musculus rectus dorsalis eye muscle (right eye). **MF:** Muscle fiber, **P:** Perimisyum, Triple.

It was determined that the striated muscle fibers of Tuj sheep in the study and control groups were located in the periphery, were multinucleated, and showed transverse striations in the form of regular bands (Figure 3). In addition, no histopathological findings were found in all images

١١٩ of both the right eye muscles and the left eye muscles of the study and control groups. It was
١٢٠ observed that all eye muscles were compatible with normal muscle tissue (Figure 4).



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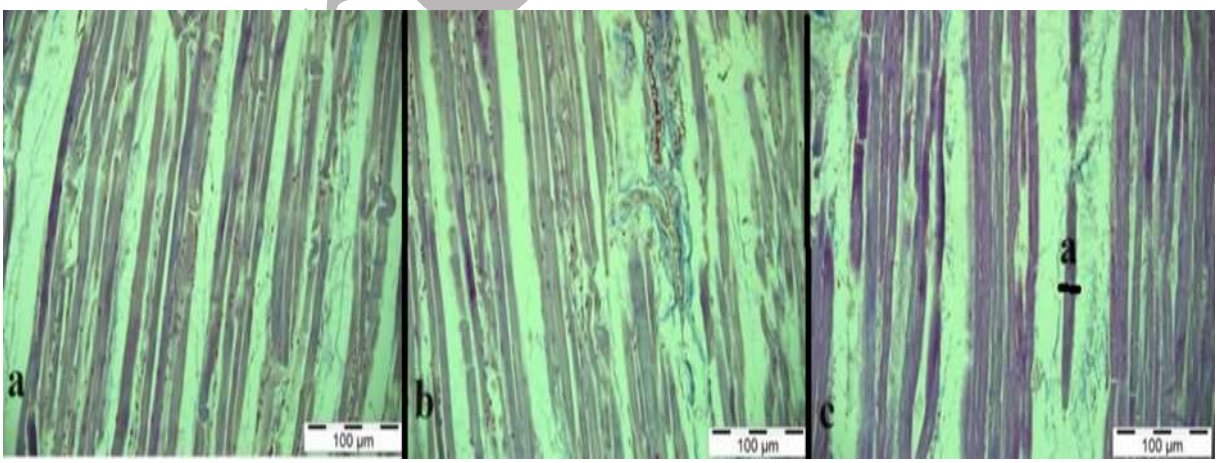
١٢٢ **Figure 3.** Eye muscles of Tuj sheep **a)** Study group, right eye, musculus retractor bulbi eye
١٢٣ muscle, (right eye) **b)** Control group, musculus retractor bulbi eye muscle (right eye). **Arrow:**
١٢٤ Peripherally located skeletal muscle cell nucleus, -- Muscle fiber thickness, Triple.



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١٢٦ **Figure 4.** Tuj sheep eye muscles **a)** Control group, musculus rectus lateralis eye muscle
١٢٧ (right), **b)** Control group, musculus rectus medialis eye muscle (right), **c)** Study group, right
١٢٨ eye musculus rectus ventralis eye muscle (right). **a:** Muscle fiber thickness, Triple.

The average values of musculus rectus dorsalis and musculus rectus medialis muscle wire thickness in the study group were significantly higher than the control group ($p<0.05$). There was no significant difference between the average muscle fiber thickness values of the musculus rectus ventralis, musculus retractor bulbi, musculus rectus lateralis and musculus obliquus dorsalis study groups and the average muscle thickness values of the control group ($p>0.05$) (Table 3).

Table 3. Results of Tuj sheep eye muscle fiber thickness in groups

	Control Group			**Study Group**			**P value**
Eye muscle	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	
MRD	60,00	114	94,73±18,50	114	300	183,00±68,00	**0,007**
MRV	60,30	115.26	89,62±16,49	96.75	120	89,62±16,48	0,351
MRM	67,08	126.58	95,09±21,97	78.23	156	109,10±34,71	**0,009**
MRL	61,19	109.50	90,23±21,26	67.08	138.5	105,44±26,18	0,142
MOD	60,00	169.70	105,95±33,73	96.00	193.49	142,31±37,91	0,235
MOV	60,06	168,90	104,55±23,43	95.00	190.64	141,32±33,22	0,230
MRB	72,00	96.74	85,93±11,81	78	150.48	112,82±21,94	0,062

MRD: Musculus rectus dorsalis, **MRV:** Musculus rectus ventralis, **MRM:** Musculus rectus medius, **MRL:** Musculus rectus lateralis, **MRB:** Musculus retractor bulbi, **MOD:** Musculus obliquus dorsalis, **MOV:** Musculus obliquus ventralis

4. Discussion

The eye muscles of the roe are defined as musculus rectus dorsalis, musculus rectus ventralis, musculus rectus lateralis, musculus rectus medialis, musculus obliquus dorsalis, musculus obliquus ventralis. The length of these muscles was measured as 36.51±2.55 mm, 34.09±3.99 mm, 35.39±2.73, 31.40±3.77 mm, 42.19±3.11, 36.06±3.10 mm, respectively (6). In Tuj sheep, the length of musculus rectus dorsalis was found to be 25.40 ± 3.91 mm on the right side and 34.50 ± 2.70 mm on the left side. The length of the same muscle was measured as 25.40 ± 3.91 mm and 34.50 ± 2.70 mm in the control and study groups, respectively. When we interpret the results, it is understood that the values on the left side and in the study group are significantly higher. According to this result, we can say that superoxide dismutase has a positive effect on the development of the musculus rectus dorsalis. Similar to Roe (6), the longest muscle in the control group was musculus obliquus dorsalis, whereas in the study group, the longest muscle was musculus rectus dorsalis. The width of these muscles in the Roe deer was reported as 9.42±0.57 mm, 9.23±0.80 mm, 8.99±0.52 mm, 9.22±0.80 mm, 5.77±0.56 mm, 8.70±0.73 mm, respectively (6). Similar to Roe deer, the largest muscle in the study group was musculus rectus dorsalis, whereas in the control group, the largest muscle was musculus rectus lateralis. In addition, the length of the musculus retractor bulbi was calculated as 30.65 ± 2.40 mm in the roe deer (6). In Tuj sheep, the longest muscle in the control group was musculus obliquus dorsalis (34.43 mm) and the shortest muscle was musculus retractor bulbi (21.74 mm). In the study group, the longest muscle was musculus rectus dorsalis (34.5 mm) and the shortest muscle was musculus retracor bulbi (22.27 mm). It is thought that this difference arises from the fact that the superoxide dismutase enzyme interacts differently on each eye muscle.

The longest and narrowest muscle among the eye muscles in the roe deer was determined to be musculus obliquus dorsalis (6). Among the rectus group muscles in New Zealand rabbits,

musculus rectus medialis (16.01±2.77 mm) was found to be the shortest muscle and musculus rectus lateralis (1.39±0.34 mm) was found to be the thinnest muscle. Among the intraorbital eye muscles in the New Zealand rabbit, musculus obliquus dorsalis (20.76±2.38 mm) was found to be the longest muscle and the thinnest muscle (1.16±0.13 mm) (5).

In cross-sectional histological images of the eye muscles, it is seen that the striated skeletal muscle system consists of muscle fibers that are surrounded by the endomysium and form muscle bundles. These bundles are organized into groups by epimysium (10, 11). There are transverse lines and A and I bands in longitudinal sections. Each muscle fiber is supported around it by a connective tissue called endomysium. These fibers come together to form long bundles called fascicles. Fascicles are also surrounded by loose connective tissue called perimysium. The majority of muscles are formed by the coming together of many fascicles and are surrounded by a thick and dense connective tissue sheath with collagen content called epimysium (12). In our study, it was observed that the musculus rectus dorsalis, musculus rectus ventralis, musculus rectus medialis, musculus rectus lateralis, musculus obliquus dorsalis, musculus obliquus ventralis and musculus retractor bulbi muscles obtained from Tuj sheep in the study and control groups consisted of a striated skeletal muscle system and showed transverse striations.

In mammals and winged animals, it is seen that there are many peripheral, round nuclei located in the periphery of the fibra muscularis (13, 14, 15). In our study, it was determined that the striated muscle fibers of the tissue samples obtained from Tuj sheep in the study and control groups and stained with Triple were located in the periphery, were multinucleated, and showed transverse stripes in the form of regular bands.

In our study, according to the muscle thickness measurement results of the study group and the control group, the average muscle fiber thickness values of the musculus rectus dorsalis and musculus rectus medialis study group were found to be significantly higher than the control group ($p<0,05$). In addition, no significant difference was detected between the average muscle fiber thickness values of the musculus rectus ventralis, musculus rectus medialis, musculus rectus lateralis and musculus obliquus dorsalis study groups and the average muscle fiber thickness values of the control group ($p>0,05$). It has been shown that vascular smooth muscle cells synthesize large amounts of Superoxide dismutase. These cells are thought to be the main source of the enzyme in the vascular wall (16).

Regulation of superoxide dismutase levels may play a particularly important role in the pathogenesis of vascular-related diseases such as atherosclerosis, coronary artery diseases, hypertension, diabetes, and ischemia/reperfusion injury (17, 18, 19). As a result of histological evaluation, it was determined that the average values of musculus rectus dorsalis and musculus rectus medialis muscle wire thickness of the sheep in the study group treated with superoxide dismutase were significantly higher than the control group. The new generation antioxidant feed additive rich with new superoxide dismutase at the level of 30g/ton in the study group diet did not have a significant effect on other eye muscles, but it had a positive effect especially on the musculus rectus medialis, which was clearly seen in both anatomical and histological evaluation. It is thought that the presented study will contribute to intraocular eye surgery operations and research relating eye diseases to nutrition.

Conflict of interest

The authors have declared no conflicts of interest.

Author Contributions

GKD, SEY, EKS, MÖ and TŞ conceived and planned the experiments. GKD and SEY carried out the research. GKD, SEY and EKS planned and carried out the study. GKD, SEY, EKS, MÖ and TŞ contributed to sample preparation. GKD, SEY, EKS, MÖ and TŞ contributed to the interpretation of the results. GKD took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

To conduct this study, necessary permissions were first obtained from Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK/2023-129).

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