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Original Article

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

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ABSTRACT

In this 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. 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 the anatomical evaluation, the longest muscle in the control group was *musculus obliquus dorsalis* (34.43 mm), while the shortest muscle was musculus retractor bulbi (21.74 mm). In the study group, the longest muscle was *musculus rectus dorsalis* (34.5 mm), while the shortest muscle was *musculus retracor bulbi* (22.27 mm). Mallory's modified triple staining (Triple) was applied for histolohical examination. In this 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 both 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 evaluations was that superoxide dismutase had a positive effect on the *musculus rectus medialis*, one of the eye muscles. It is thought that the present study will contribute to intraocular eye surgery operations and research relating eye diseases to nutrition.

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1. Introduction

The homeland of Tuj sheep is Turkey. They are raised in the provinces of Kars, Ardahan, and Iğdır. This combination is efficient. It produces meat, wool, and milk. It has a small, bright white body. Black pigment appears around the eyes, mouth, and 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. Superoxide dismutase has been observed to have both protective and therapeutic properties (2).

There are four rectus muscles that control eye movement: dorsal, ventral, lateral, and medial. These muscles are responsible for turning the eyeball upward, downward, inward, and outward. *Musculus obliquus dorsalis* rotates the eyeball downward and outward, while *musculus obliquus ventralis* turns it upward and outward. The *musculus retractor bulbi* draws the eyeball back into the orbita (3, 4).

Studies on eye muscles have been conducted in various animal species (5–8). However, it has been observed that studies on eye muscles are limited in terms of the species. In this sense, the aim of this study was to address this deficiency and evaluate the effect of superoxide dismutase on eye muscle anatomy and histology.

2. Materials and Methods

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 lasted 60 days. During the fattening period, the sheep were fed 700 g of concentrated feed, 225 g of fresh sugar beet pulp, and 200 g of wheat straw twice daily (at 08.00 and16.00). Also, water was offered ad-libitum. While no additive was included in the control's group diet, a new-generation antioxidant feed additive rich in novel superoxide dismutase was incorporated into the study group's diet at a concentration of 30g/ton. The feed additive product was sourced from a private commercial company (MeloFeed®, Lallemand Animal Nutrition, Canada).

2.2. Anatomical procedures

In this study, 16 eye muscles from a total of eight male Tuj breed sheep were examined over a period of approximately 2-3 months. Animals that had reached the desired maturity were slaughtered and subsequenly transported to the Anatomy department laboratory. After skin dissection, the orbita was carefully accessed from the medial and lateral parts of the eyes. The eye, along with its surrounding auxiliary structures, awere removed from the eyeball. 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. The length, width, and thickness of each eye muscle were measured using a digital caliper. Scientific terms were applied according to N.A.V. (9).

2.3. Histological procedures

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

2.4. Statistical analysis

The mean and standard deviation values of all measurements obtained from anatomical examinations, along with the differences between the control and study groups, were determined using the Independent Samples T-test in the SPSS software package program (20.0 version, IBM Corp., Armonk, NY, US). The statistical significance level was set at $p<0.05$.

3. Results

3.1. Anatomical results

It was determined that the *nervus opticus* followed a course toward the *musculus retractor bulbi*. The *musculus retractor bulbi* was located between the *musculus rectus ventralis* and the *musculus rectus lateralis*. *Musculus rectus dorsalis* and *musculus obliquus dorsalis* originated from a common root (Figure 1).

In the control group, the longest muscle was *musculus obliquus dorsalis* (34.43 mm), while the shortest muscle was *musculus retractor bulbi* (21.74 mm). In the study group, the longest muscle was *musculus rectus dorsalis* (34.5 mm), while the shortest muscle was *musculus retracor bulbi* (22.27 mm). Regarding directional comparisons, the longest muscle on the right side was *musculus obliquus dorsalis* (34.43 mm), whereas on the left side, it was *musculus rectus dorsalis* (34.5 mm).

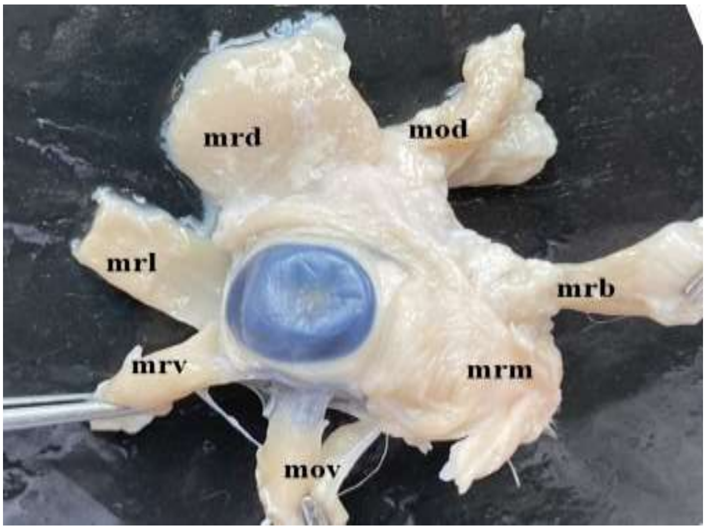

Figure 1. Eye muscles in Tuj sheep. **mrd:** musculus rectus dorsalis, **mod:** musculus obliquus dorsalis, **mrv:** musculus rectus ventralis, **mrl:** musculus rectus lateralis, **mov:** musculus obliquus ventralis, **mrb:** musculus retractor bulbi, **mrm:** musculus rectus medialis.

In the control group, the widest muscle was *musculus rectus lateralis* (11.95 mm), while 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), while the narrowest was *musculus obliquus dorsalis* (5.26 mm). In the control group, the thickest muscle was identified as *musculus rectus ventralis*, while the thinnest was *musculus rectus dorsalis*. In the study group, the thickest muscle was *musculus rectus medialis*, while the thinnest was *musculus rectus lateralis*. Statistical data on the eye muscles in both control and study groups are presented in Table 1. Directional evaluation of the same animals is shown in Table 2.

3.2. Histological results

In Tuj sheep from both 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* exhibitted normal histological structure. Upon examination of the interstitial connective tissue of the muscles in Tuj sheep from both the study and control groups, no changes were observed in the connective tissue cells or in the distribution of connective tissue fibers. Muscle fibers were observed to be surrounded by connective tissue containing blood vessels and nerves. In cross-sectional histological images, the striated skeletal muscle system consisted of muscle fibers that were surrounded by the endomysium, forming distinct muscle bundles. The muscle bundles were observed to be grouped by the epimysium.

It was determined that transverse lines and A and I bands were present in the longitudinal sections (Figure 2).

It was determined that the striated muscle fibers of Tuj sheep in both the study and control groups were located peripherally, were multinucleated, and showed transverse striations in the form of regular bands (Figure 3). In addition, no histopathological findings were observed in any of the images of the right or left eye musclesin both the study and control groups. It was observed that all eye muscles were compatible with normal muscle tissue (Figure 4).

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

4. Discussion

The roe’s eye muscles are defined as *musculus rectus dorsalis, musculus rectus ventralis, musculus rectus lateralis, musculus rectus medialis, musculus obliquus dorsalis,* and *musculus obliquus ventralis*. The length of these muscles were measured as 36.51±2.55 mm, 34.09±3.99 mm, 35.39±2.73 mm, 31.40±3.77 mm, 42.19±3.11 mm, and 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.

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.

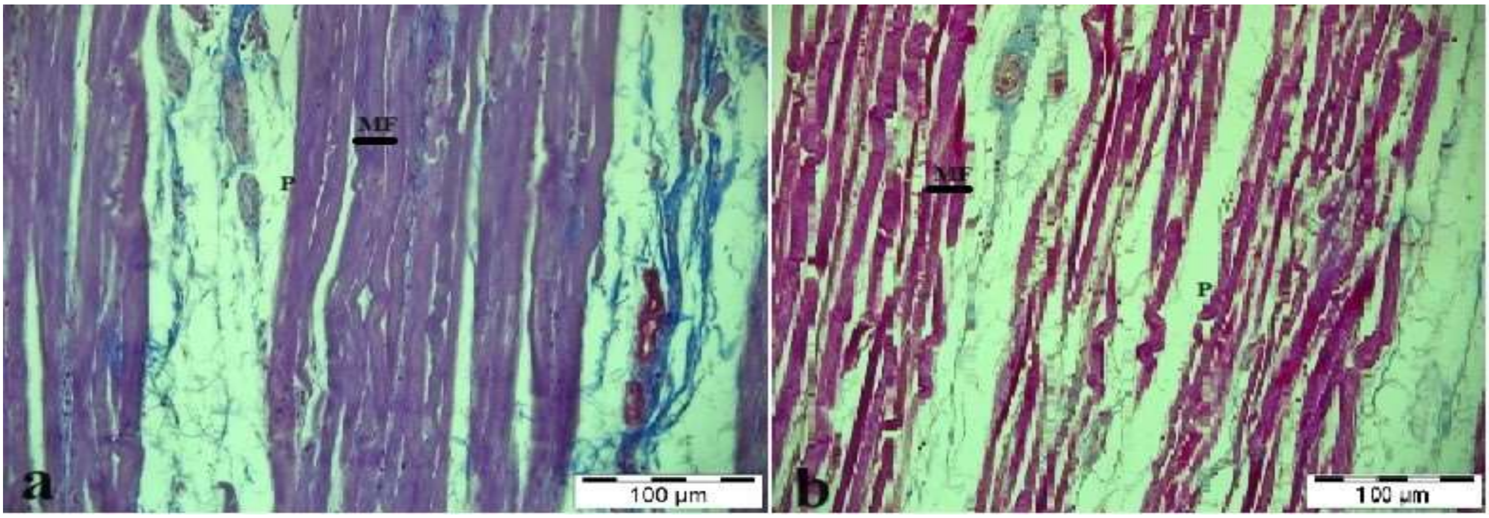

Figure 2. Tuj sheep eye muscles **a)** Study group, musculus rectus dorsalis eye muscle (right eye), **b)** Control group, musculus rectus dorsalis eye muscle (right eye). **MF:** Muscle fiber, **P:** Perimisyum, Triple.

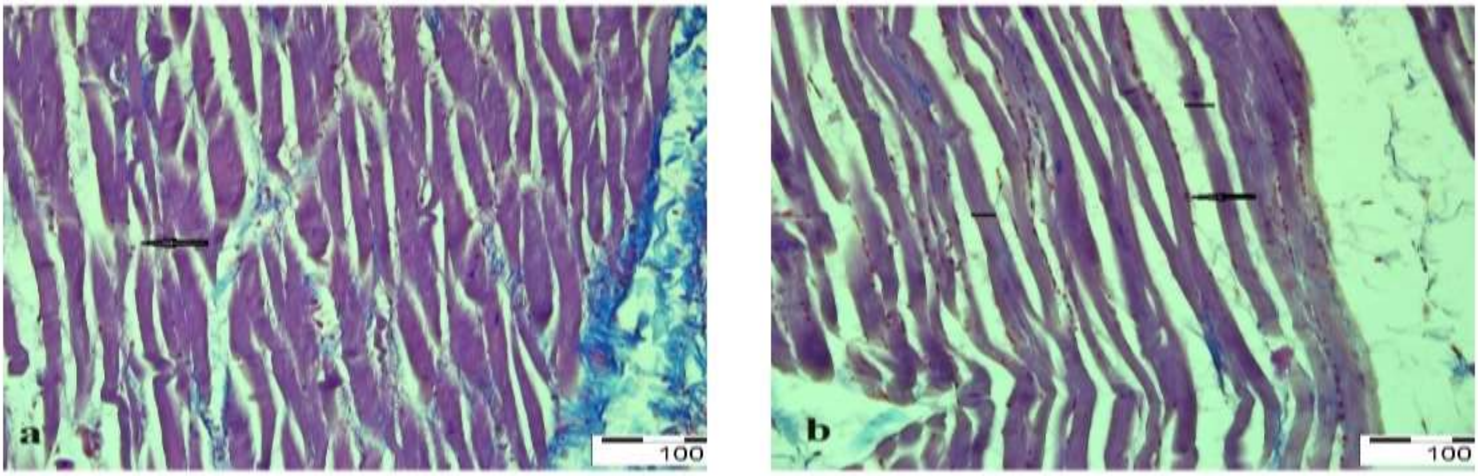

Figure 3. Eye muscles of Tuj sheep **a)** Study group, right eye, musculus retractor bulbi eye muscle, (right eye) **b)** Control group, musculus retractor bulbi eye muscle (right eye). **Arrow:** Peripherally located skeletal muscle cell nucleus, -- Muscle fiber thickness, Triple.

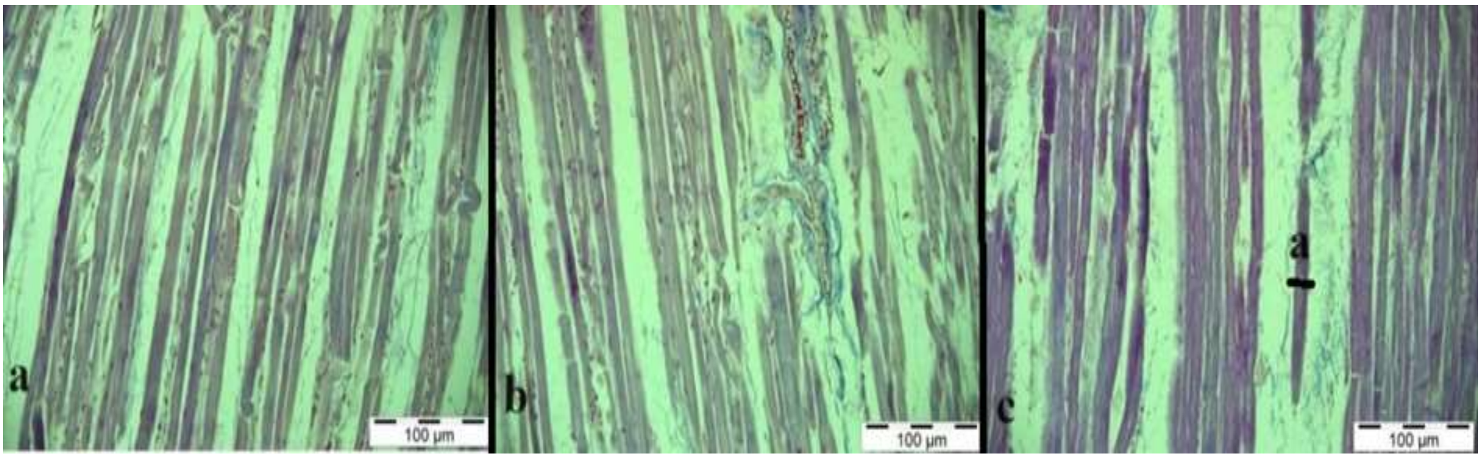

Figure 4. Tuj sheep eye muscles **a)** Control group, musculus rectus lateralis eye muscle (right), **b)** Control group, musculus rectus medialis eye muscle (right), **c)** Study group, right eye musculus rectus ventralis eye muscle (right). **a:** Muscle fiber thickness, Triple.

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.

The same measurements were recorded in the control and study groups, respectively. When interpreting the results, it is evident that the values on the left side and in the study group are significantly higher.

According to these results, it can be inferred that superoxide dismutase exerts a positive effect on the development of *musculus rectus dorsalis*. Similar to the Roe (6), the longest muscle in the control group was *musculus obliquus dorsalis*, whereas in the study group, it was *musculus rectus dorsalis*. The width of these muscles in the roe deer were 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, and 8.70±0.73 mm, respectively (6). Similar to the roe deer, the largest muscle in the study group was *musculus rectus dorsalis*, whereas in the control group, it 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), whereas shortest was *musculus retractor bulbi* (21.74 mm). In the study group, the cross-sectional histological images of the eye muscles, the striated skeletal muscle tissue appears as organized muscle fibers surrounded by endomysium, which group together to 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 surrounded by a connective tissue called endomysium. These fibers come together to form long bundles called fascicles. Fascicles are also surrounded by loose connective tissue known as perimysium. The majority of muscles are composed of many fascicles, which are surrounded by a thick and dense connective tissue sheath rich in collagen, called epimysium (12).

In this 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 both the study and control groups consisted of striated skeletal muscle tissue and showed transverse striations.

In mammals and winged animals, many round peripheral nuclei are observed at the edge of the fibra muscularis (13-15). In this study, it was determined that the striated muscle fibers in tissue samples obtained from Tuj sheep in both the study and control groups and stained using the Triple method were peripherally located, multinucleated, and showed transverse striations arranged as regular bands.

In this study, according to muscle thickness measurement from the study and control groups, the average muscle fiber thickness values for *musculus rectus dorsalis* and *musculus rectus medialis* were found to be significantly higher in the study group than in 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* in the study group and those in the control group ($p>0,05$). Vascular smooth muscle cells have been shown to synthesize significant amounts of superoxide dismutase and are considered the main source of this enzyme in the vascular wall (16).

Regulation of superoxide dismutase levels may play an important role in the pathogenesis of vascular-related diseases, including atherosclerosis, coronary artery diseases, hypertension, diabetes, and ischemia/reperfusion injury (12, 17). Histological evaluation revealed that the average muscle fiber thickness of the *musculus rectus dorsalis* and *musculus rectus medialis* muscle in sheep treated with superoxide dismutase was significantly higher than that of the control group. The new-generation antioxidant feed additive, enrich with superoxide dismutase at a concentration of 30g/ton in the study group diet, did not significantly effect other eye muscles. However, it showed a particularly positive effect on the *musculus rectus medialis*, as evidenced by both anatomical and histological evaluations. The findings of the present study are expected to contribute intraocular surgical procedures and to research investigating the link between eye diseases and nutritional interventions.

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Authors' Contribution

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.

Ethics

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

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

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

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