Effects of short-term transportation and lairage time on meat quality, *CAST*, *CAPN1*, *CAPN3*, *HSPA1A* gene expression levels and myofibrillary structure of *M. longissimus dorsi* of Kıvırcık breed sheep

Nüket BİLGEN[1,a,✉], Ahmet CEYLAN[2,b], Funda YILMAZ EKER [3,c], Esra AKKAYA[3,d], İsmail Şah HAREM[4,e] Bengi ÇINAR KUL[1,f], Enver Barış BİNGÖL[3,g], Feraye ESEN GÜRSEL[5,h], Atila ATEŞ[5,i], Doğukan ÖZEN[6,j] Ömer ÇETİN[7,k], Alev Gürol BAYRAKTAROĞLU[2,l], Tolga KAHRAMAN[3,m]

[1]Ankara University, Faculty of Veterinary Medicine, Department of Genetics, Ankara, Türkiye; [2]Ankara University, Faculty of Veterinary Medicine, Department of Histology Embryology, Ankara, Türkiye; [3]İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, İstanbul, Türkiye; [4]Harran University Faculty of Veterinary Medicine, Department of Histology Embryology, Urfa, Türkiye; [5]İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Biochemistry İstanbul, Türkiye; [6]Ankara University, Faculty of Veterinary Medicine, Department of Biostatistics, Ankara, Türkiye; [7]İstanbul Rumeli University, Faculty of Health Sciences, Department of Nutrition and Dietetics, İstanbul, Türkiye

[a]ORCID: 0000-0003-2324-7965; [b]ORCID: 0000-0001-5878-8775; [c]ORCID: 0000-0003-4315-5363; [d]ORCID: 0000-0002-2665-4788 [e]ORCID: 0000-0002-5947-4118; [f]ORCID: 0000-0002-8955-0097; [g]ORCID: 0000-0002-6452-4706; [h]ORCID: 0000-0002-1595-2827 [i]ORCID: 0000-0002-9013-930X; [j]ORCID: 0000-0003-1943-2690; [k]ORCID: 0000-0002-5269-090X; [l]ORCID: 0000-0001-8667-0184 [m]ORCID: 0000-0003-4877-6951

ARTICLE INFO

Article History
Received : 21.01.2022
Accepted : 17.08.2022
DOI: 10.33988/auvfd.1061335

Keywords
Calpain-calpastatin
Meat tenderness
Myofibrillary structure
Sheep
Transportation

✉Corresponding author
nbilgen@ankara.edu.tr

How to cite this article: Bilgen N, Ceylan A, Yılmaz Eker F, Akkaya E, Harem İŞ, Çınar Kul B, Bingöl EB, Esen Gürsel F, Ateş A, Özen D, Çetin Ö, Bayraktaroğlu AG, Kahraman T (2023): Effects of short-term transportation and lairage time on meat quality, *CAST*, *CAPN1*, *CAPN3*, *HSPA1A* gene expression levels and myofibrillary structure of *M. longissimus dorsi* of Kıvırcık breed sheep. Ankara Univ Vet Fak Derg, 70 (4), 401-412. DOI: 10.33988/auvfd.1061335.

ABSTRACT

The objective of this study was to examine the effect of short-term transportation and lairage time on meat quality, myofibrillary structure, calpastatin (*CAST*), μ-calpain (*CAPN1*), calpain 3 (*CAPN3*), and heat shock protein 70 (*HSPA1A*) expression levels of *M. longissimus dorsi* of Kıvırcık breed sheep (n=40). Meat quality was evaluated by muscle glycogen amounts, pH, temperature, cooking loss, water holding capacity, shear force, instrumental color, sarcomere length, and organoleptic properties. Cellular changes in the muscle during the aging process were scanned by electron microscopy and *CAST*, *CAPN1*, *CAPN3*, *HSPA1A* expression levels were measured to reveal the association on meat tenderness. As a result, a positive effect of long lairage time groups was observed in the pH, glycogen, SF values, and organoleptic evaluation. *CAST*, *CAPN1, CAPN3* expression showed no difference, however, *HSPA1A* showed a significant difference in the aging process. In conclusion, genotypic differences, their effect on gene expression, and protein level on meat quality should be further investigated.

Introduction

Animal welfare promotes living conditions that are free from diseases, injury, malnutrition, pain, distress, and hunger (44). Animal welfare includes not only the physical health and biological conditions of the animal but also the affective states of the animal that improve and standardize meat quality (34, 43). In animal husbandry, transportation is one of the most important factors affecting the welfare and health of the animals before slaughter (1).

According to animal welfare guidelines, it is recommended to choose the closest possible slaughterhouse location to the farm or a maximum of 8 h of transport called short term transportation (33). Schaefer et al. (39) stated that transportation that is not performed under appropriate conditions decreases meat quality. Animals should be rested at least 24 hours before slaughter to eliminate the negative effects of transportation (29). The lairage period has considerable attention for recovering from stress experienced during the transport (21). Mounier et al. (35) reported that lairage time could allow animals to recover muscle glycogen concentrations. Terlouw et al. (41) indicated that 24-hour lairage could be returned conditions before shipping.

Several studies regarding the effect of transport and lairage time on meat quality were carried out by Ekiz et al. (13), Diaz et al. (12), Najafi et al. (36) in lambs and Ferreira et al. (16), Gallo et al. (18), Maria et al. (34) in cattle. Consumer acceptance of meat depends on its quality characteristics including color, tenderness, flavor, and water holding capacity (WHC) can be affected by proteolytic degradation of the myofibrillar protein (31). In the proteolytic enzymes, calpastatin (*CAST*), μ-calpain (*CAPN1*), calpain 3 (*CAPN3*) and heat shock protein (*HSPA1A*) *g*enes are responsible for all biochemical reactions in the conversion of muscle to meat (5). However, the effects of *CAST*, *CAPN1*, *CAPN3*, *HSPA1A* expression levels on aging and quality have not been extensively investigated.

The aim of the study was to determine the effect of short-distance transport and lairage time on glycogen content and meat quality of Kıvırcık breed sheep and association with expression levels of *CAST*, *CAPN1*, *CAPN3*, and *HSPA1A g*enes. And also, the changes in the muscle cells during the aging, the sarcomere lengths, and contractile band shapes were examined, the histological structure of meat was scanned by scanning electron microscopy.

Material and Methods

Animals and experimental design: In the study, 40 male Kıvırcık breed yearling lambs were used. Pregnant sheep were taken to the birth chamber 2 weeks before the birth. After the birth, sheep and lambs were placed in individual compartments for three weeks. Lambs were weaned on day 75 and were fed with ad-libitum concentrate (16% HP) and roughage until slaughter time. Kıvırcık lambs were raised under the same breeding program until the age of about 1 year. The care and feeding of the lambs from birth to slaughter time were carried out in İstanbul University, Faculty of Veterinary Medicine, Training and Research Farm Sheep Unit (The research protocol of the study was approved by the Animal Experiment Local Ethics Committee of İstanbul University 2016/15). Yearling lambs were weighted around 46 ± 2 kg.

Pre-slaughter and slaughter process: Yearling lambs were transported from the Research Farm Sheep Unit to the slaughterhouse in a 1.90 m × 4 m truck body and a maximum of 20 animals were transported at a time. To eliminate the effect of large temperature variation during transport, the study was carried out at seasonal temperatures of 15-20°C. Transportation time was set at 3 hours. Yearling lambs were separated into 4 groups, consisting of 10 individuals. The first group was slaughtered immediately after transportation (LT_0), the second group was slaughtered after 12 hours lairage time (LT_{12}), and the third group was slaughtered after 24 hours lairage time (LT_{24}). The fourth group, kept as a control (C), was treated according to guidelines (21), where the animals were rested for 1 week at the slaughterhouse resting paddocks and then slaughtered.

Slaughtering was carried out in accordance with animal welfare in İstanbul University-Cerrahpaşa Faculty of Veterinary Medicine Education Research Slaughterhouse. Animals were electrically stunned at 220-250 voltage, 1.0–1.3 amps, and 1–3 second, stunning tongs applied on both sides of the head. The bleeding process was followed by skinning and removal of the internal organs, which took approximately 30 ± 5 minutes.

Carcass sampling and meat quality analysis: Samples, which were immediately taken after slaughtering (*M. longissimus dorsi* (LD) of the right side of carcasses) muscles, were used for biochemical, histological, and molecular analysis. The left side of carcasses were stored at the cold chain until 24 hours of postmortem and then used for meat quality (cooking loss, water holding capacity, shear force, sarcomere length, and color) and sensorial analyses. Samples were vacuum-packed in Cryovac barrier bags (Cryovac Sealed Air Corp., New Jersey, USA) and stored in cold storage (0-4°C) for 10 days. Molecular analysis of samples was carried out on days 0 and 2, biochemical and histological analysis were carried out on days 0, 2, 5, and 10. Meat quality and organoleptic analyzes were performed on days 2, 5, and 10.

Meat quality analysis: Muscle glycogen determination was performed using the Glycogen Assay Kit (Biovision, USA #K646-100) following the manufacturer's instructions for the colorimetric assay.

The temperature and pH were measured of the samples taken from the upper surface of the LD at the 1st hour of the postmortem stage and at different control times (1^{st}, 3^{rd}, 6^{th}, 12^{th}, 24^{th} hours) within the first day. A digital thermometer (Hanna HI 99163, USA) was used for

DOI: 10.33988/auvfd.1061335

temperature measurement. The electrodes of pH and temperature devices were placed near the center of three different points of the muscle and calculated by taking the average of the determined values (27).

Cooking loss (CL) analysis was carried out with 50 g meat samples, which were cooked in a mini water bath at 100°C for 10 minutes. After cooking, the meat was dried and weighed (45). The cooking loss was calculated by the following formula:

Cooking Loss: 100-100 × weight after cooking / weight of raw meat

The percentage of free liquid was evaluated as a measure of water holding capacity (WHC) by the filter press method described by Vural and Öztan (47). The outline area of the expressible juice and the meat film were traced, and two areas were measured using AUTOCAD 2008 (Apso Ltd, UK). The water retention capacity was calculated by the following formula:

Water Retention Capacity: Spread Area of Meat (cm^2) / Total Area (cm^2)

Shear force (SF) analysis of LD muscles was performed by Warner-Bratzler shear force texture analyzer (Instron, model 3343, UK). The meats taken on the relevant days of the enclosure were sliced 1 × 1 cm thick and then cooked in a water bath at 100°C for 10 minutes. For each sample, the highest force (kg / cm^2) and force x time graph of the Warner-Bratzler blade cutting sequence were recorded to a computer using the BlueHill 2 operating system (46). SF value was determined by calculating the arithmetic means of the multiple readings obtained from the samples.

Instrumental color measurements of the muscles at five different locations on each meat sample were averaged at each sampling day, immediately after opening each package in terms of CIE *L** (lightness), *a** (redness), and *b** (yellowness) values by measuring with a Colorflex HunterLab Spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA). Color values were evaluated using diffuse illumination (D65 2° observer) with an 8 mm viewing aperture and a 25 mm port size with the specular component excluded from the colorimeter (2).

Histological analysis and Scanning Electron Microscopy (SEM): To determine the sarcomere length (SL), 1 cm^3 of tissue was collected and fixed in 10% neutral buffered formalin. After following a routine histological process, the tissue was embedded in paraffin. Masson's Trichrome staining was performed on the sections and photographed by a microscope (Leica DM2500, Leica Microsystems GmbH, Wetzlar, Germany) with a digital camera (Leica DFC450, Leica Microsystems GmbH, Wetzlar, Germany). Each sample was evaluated by measuring 10 different sarcomere lengths (9).

For SEM analysis, the samples were rinsed with physiological saline then fixed in FAA solution (10% formaldehyde, 5% acetic acid, and 85% alcohol) solution. Following dehydration protocols and coated with hexamethyldisilazane (HMDS), they were placed in copper containers. Samples were sent to the Department of Histology and Embryology of the Faculty of Veterinary Medicine of Harran University and visualized under the scanning electron microscope (EVO50, Zeiss, Germany).

Gene expression analysis: Total RNA were isolated using 100-200gr pieces that were cut from the lateral ½ part of the LD muscle. Samples were homogenized with liquid nitrogen and mixed with PureZol (Biorad, US). An additional step was applied to remove the lipid in the meat, all samples were stored at -80°C overnight in PureZol solution (Biorad, US). On the second day, tissue residues were removed by 10 min centrifuge at 15000 rpm at +4°C. Then PureZol instructions were followed. Obtained RNAs were quantified spectrophotometrically, and DNase treated (5U/ul). Total cDNA were synthesized using 100 ng/ul RNA template by iScript™ Reverse Transcription Supermix **(**Biorad, US). Amplification program of the cDNA was 50°C for 2 min and 95°C for 2 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The primers for target genes were designed specifically to the exon-exon junction points on the mRNA reference sequences using Primer3 website (Table 1) and GAPDH used as housekeeping gene. Real-time PCR was performed using SsoAdvanced Universal SYBR Green Supermix (Biorad, US) with 3 technical replicas by CFX Connect real-time PCR System (BioRad, US). The obtained cycle threshold values (Cycle Threshold, CT) were normalized according to housekeeping and differences between the groups were analyzed.

Sensory analysis: Organoleptic properties of LD samples were evaluated by a panel of ten trained participants of the staff of İstanbul University-Cerrahpaşa, Food Hygiene and Technology Department, who had previously participated in training sessions to become familiar with the sensory characteristics of meat (23, 25). The panelists were requested to score the sensory attributes (color intensity, color hue, odor intensity, tenderness, chewiness, juiciness, flavor intensity, flavor quality, and general appearance acceptability) using an unstructured 10-point line scale (0: extremely unacceptable, 1: unacceptable, 5: between acceptable, 10: extremely acceptable).

Table 1. Designed oligonucleotides sequences, amplicon length, annealing temperatures, and accession numbers.

	Oligonucleotides sequence	Amplicon length	Annealing temperature	Accession
CAST_F	CTGTGGCAGAGGATGTGCCT	109bp	62ºC	NM_001009788.1
CAST_R	GCCAAGGCTTCCACAGCATC			
HSPA1A_F	CCTGTTCGAGGGCATCGACT	193bp	60ºC	NM_001267874.1
HSPA1A_R	CAGCAGCTTCTGCACCTTGG			
GAPDH_F	GTCAGCAATGCCTCCTGCAC	138bp	60ºC	NM_001190390.1
GAPDH_R	GGAAGGGCCATCCACAGTCT			
CAPN1_F	AGTGGGTGGATGTGGTGGTG	75bp	60ºC	NM_001127267.1
CAPN1_R	CTTGGGCAGAGTGCACGAAC			
CAPN3_F	AAGCCTCCAGGCACCTCATC	183bp	60ºC	NM_001009212.1
CAPN3_R	CTGGCCTGTGGATAGCCGAA			

Table 2. Definition of the attributes used in the sensory analysis of meat samples.

Attribute	Definition
Color intensity	Clear, strong red color
Color hue	Deviation of color from yellow/red to red/green
Odor intensity	The intensity of sum of all odors related to sheep
Tenderness	The force required to bite through the sample
Chewiness	Time and numbers of chewing required to masticate the sample ready for swallowing
Juiciness	Perception of water content in the sample after 3-4 chewing
Flavor intensity	Level of the overall flavor in the mouth
Flavor quality	Flavor experienced prior to swallowing related with sheep
General appearance acceptability	Overall liking from the panelists

Primarily, the panelists were trained in three separate sessions for approximately 2 hours for the evaluation of each selected attribute (Table 2). Training sessions were conducted to acquaint panelists with the products and attributes to be evaluated and were followed by an open discussion session to familiarize panelists with the attributes and the scale to be used.

Following that a kitchen-type oven (Siemens, Germany) was used to cook meat samples at 200°C until the internal temperature reached 80°C. Samples were then served immediately to the panelists where they were seated in individual booths in a temperature and light-controlled room (fluorescent lighting of 2000 lx; Philips 40W Cool White). Each sample was labeled, at random, with a two-digit code number and sensory analysis was performed in duplicate in two sessions (24).

Statistical analysis: Descriptive statistics were calculated for all variables measured in the study and shown as "Arithmetic Mean ± Standard Error of the mean". Prior to the significance tests, the data were checked by Shapiro Wilk in terms of conformity to the normal distribution from the parametric test assumptions, and Levene's test in terms of homogeneity of variances. Two-way analysis of variance was applied by using the general linear modeling technique to analyze the effect of time and group on the variables obtained. In the established model, the basic effect of "Group" and "Time" and "Group x Time" interaction terms are included. In cases where interaction terms were found to be meaningful, simple effects analysis was performed by applying Bonferroni correction to analyze the interaction terms. In cases where the interaction terms were not significant, Bonferroni corrected pairwise comparisons were performed for any significant main effect term in the model to evaluate differences among the levels within each factor. Stata 16.1 was used for data analysis. P-value <0.05 criterion was taken into consideration in all statistical decisions. In gene expression analyzes, delta-delta Ct values obtained from the groups were evaluated with Student's *t*-test and obtained values from the comparisons between groups were examined. In the calculation of fold change, a previously proposed model was used (37). Each result represents the mean of 10 independent determinations.

Results

The glycogen values were found to be the lowest, especially in the groups with high lairage time (LT_{24}) and C, 4.33 and 4.21 respectively, whereas LT_0 group, the highest glycogen level was recorded with 5.62 (Table 3). The lowest pH values were determined in the LT_{24} and C groups, 7.20 and 6.99 respectively (P<0.001). Besides, in

correlation with the glycogen level, pH values continued to decrease with the longer storage time (P<0.001).

SF values were low in the LT_{24} and C groups (P<0.001), and decreased during the aging period with a similar deviation among groups (P<0.001). SF analyses resulted as 6.15, 5.34, and 4.14 on the days 2nd, 5th, and 10th days, respectively (Table 3).

The pH level at 24th hours storage time of LT_{24} and C groups was on average 5.67 whereas in LT_0 and LT_{12} groups pH level at 24th hours was on average 6.10 (Table 4).

Table 3. Mean values, standard errors, and significant interactions of biochemical, histological, and quality characteristics of sheep meats subjected to short-term transportation and lairage time.

Attribute	Time (day)	C	LT_0	LT_{12}	LT_{24}		Significance		
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	LS Mean ± SE	G	T	G x T
CL	2	39.14 ± 0.79	39.12 ± 0.93	39.15 ± 0.66	39.05 ± 0.55	**39.11 ± 0.39**	NS	NS	NS
	5	39.31 ± 0.85	39.47 ± 0.74	39.27 ± 0.94	39.37 ± 0.87	**39.35 ± 0.39**			
	10	39.57 ± 0.67	39.59 ± 0.79	39.61 ± 0.74	39.59 ± 0.69	**39.59 ± 0.39**			
	LS Mean ± SE	**39.34 ± 0.45**	**39.39 ± 0.45**	**39.35 ± 0.45**	**39.33 ± 0.45**				
WHC	2	36.42 ± 1.48	36.46 ± 1.65	36.44 ± 1.54	36.43 ± 0.77	**36.44 ± 0.69** [a]	NS	<0.001	NS
	5	28.73 ± 1.89	28.76 ± 1.75	28.75 ± 1.54	28.74 ± 1.29	**28.74 ± 0.69** [b]			
	10	22.75 ± 0.95	22.73 ± 1.20	22.71 ± 0.83	22.78 ± 1.13	**22.74 ± 0.69** [c]			
	LS Mean ± SE	**29.3 ± 0.8**	**29.32 ± 0.8**	**29.3 ± 0.8**	**29.3 ± 0.8**				
Glycogen value	2	2.37 ± 0.2	2.94 ± 0.36	2.77 ± 0.43	2.39 ± 0.22	**2.62 ± 0.18** [b]	0.005	<0.001	NS
	5	1.92 ± 0.26	2.45 ± 0.32	2.28 ± 0.45	1.93 ± 0.19	**2.15 ± 0.18** [bc]			
	10	1.52 ± 0.12	2.2 ± 0.34	1.93 ± 0.25	1.56 ± 0.16	**1.81 ± 0.18** [c]			
	LS Mean ± SE	**2.51 ± 0.18** [B]	**3.30 ± 0.18** [A]	**3.01 ± 0.18** [AB]	**2.55 ± 0.18** [B]				
*L**	2	21.25 ± 0.67	21.30 ± 0.43	21.33 ± 1.03	21.12 ± 0.42	**21.25± 0.42** [b]	NS	<0.001	NS
	5	28.68 ± 0.82	28.63 ± 0.47	27.74 ± 1.18	28.58 ± 0.48	**28.41 ± 0.42** [a]			
	10	27.92 ± 0.63	27.89 ± 0.89	27.89 ± 1.47	27.89 ± 0.87	**27.90 ± 0.42** [a]			
	LS Mean ± SE	**25.95 ± 0.49**	**25.84 ± 0.49**	**25.65 ± 0.49**	**25.86 ± 0.49**				
*a**	2	15.53 ± 0.49	15.51 ± 0.37	15.55 ± 0.35	15.58 ± 0.51	**15.54 ± 0.19** [b]	NS	<0.001	NS
	5	15.58 ± 0.23	15.65 ± 0.41	15.64 ± 0.35	15.6 ± 0.11	**15.62 ± 0.19** [b]			
	10	16.51 ± 0.21	16.53 ± 0.52	16.49 ± 0.47	16.55 ± 0.3	**16.52 ± 0.19** [a]			
	LS Mean ± SE	**15.86 ± 0.22**	**15.90 ± 0.22**	**15.89 ± 0.22**	**15.91 ± 0.22**				
*b**	2	6.29 ± 0.46	6.33 ± 0.34	6.32 ± 0.8	6.27 ± 0.33	**6.30 ± 0.33** [c]	NS	<0.001	NS
	5	10.1 ± 0.39	10.13 ± 0.84	10.1 ± 0.56	10.12 ± 0.48	**10.11 ± 0.33** [b]			
	10	11.4 ± 0.76	11.39 ± 1.15	11.39 ± 0.61	11.4 ± 0.72	**11.4 ± 0.33** [a]			
	LS Mean ± SE	**9.26 ± 0.38**	**9.29 ± 0.38**	**9.27 ± 0.38**	**9.26 ± 0.38**				
SF	2	5.09 ± 0.16	7.31 ± 0.19	6.62± 0.21	5.58 ± 0.2	**6.15 ± 0.12** [c]	<0.001	<0.001	NS
	5	4.55 ± 0.11	6.03 ± 0.32	5.87 ± 0.31	4.91 ± 0.25	**5.34 ± 0.12** [b]			
	10	3.74 ± 0.09	4.81 ± 0.28	4.2 ± 0.28	3.8 ± 0.27	**4.14 ± 0.12** [a]			
	LS Mean ± SE	**4.46 ± 0.14** [B]	**6.05 ± 0.14** [A]	**5.56 ± 0.14** [A]	**4.76 ± 0.14** [B]				
SL	2	1.37 ± 0.04	1.28 ± 0.05	1.3 ± 0.02	1.38 ± 0.07	**1.33 ± 0.02** [b]	NS	<0.001	NS
	5	1.55 ± 0.02	1.46 ± 0.03	1.49 ± 0.01	1.5 ± 0.01	**1.5 ± 0.02** [a]			
	10	1.51 ± 0.02	1.52 ± 0.03	1.51 ± 0.03	1.53 ± 0.02	**1.52 ± 0.02** [a]			
	LS Mean ± SE	**1.40 ± 0.02**	**1.35 ± 0.02**	**1.38 ± 0.02**	**1.39 ± 0.02**				

[a, b, c]: Different letters in the same column represent statistically significant difference (P<0.05).
[A, B, C]: Different letters in the same column represent statistically significant difference (P<0.05).
NS: Not significant.
C: Control (Animals slaughtered after 1 week of lairage).
LT_0: Immediate slaughter (0 lairage time).
LT_{12}: Animals slaughtered after 12 hours of lairage.
LT_{24}: Animals slaughtered after 24 hours of lairage.
G: Group, T: Time, G x T: Group x Time, LS: Least Square.
*L**: (lightness, *a**: redness, *b**: yellowness.

Table 4. Mean values, standard errors, and significant interactions of pH and temperature changes of sheep meats subjected to short-term transportation and lairage time.

Attribute	Time (hour)	C Mean ± SE	LT_0 Mean ± SE	LT_{12} Mean ± SE	LT_{24} Mean ± SE	LS Mean ± SE	Significance G	T	G x T
pH	1	6.99 ± 0.08 $^{a\,C}$	7.46 ± 0.08 $^{a\,A}$	7.33 ± 0.05 $^{a\,AB}$	7.20 ± 0.03 $^{a\,B}$	**7.24 ± 0.03**	<0.001	<0.001	NS
	3	6.83 ± 0.07 $^{a\,b\,C}$	7.34 ± 0.06 $^{a\,A}$	7.31 ± 0.08 $^{a\,AB}$	7.05 ± 0.08 $^{a\,b\,B}$	**7.13 ± 0.03**			
	6	6.65 ± 0.03 $^{b\,B}$	7.08 ± 0.06 $^{b\,A}$	6.99 ± 0.02 $^{b\,A}$	6.92 ± 0.04 $^{b\,A}$	**6.91 ± 0.03**			
	12	6.09 ± 0.03 $^{c\,B}$	6.54 ± 0.02 $^{c\,A}$	6.32 ± 0.07 $^{c\,B}$	6.00 ± 0.03 $^{c\,B}$	**6.24 ± 0.03**			
	24	5.70 ± 0.03 $^{d\,B}$	6.14 ± 0.02 $^{d\,A}$	6.07 ± 0.03 $^{d\,A}$	5.64 ± 0.03 $^{b\,B}$	**5.89 ± 0.03**			
	LS Mean ± SE	**6.45 ± 0.02**	**6.91 ± 0.02**	**6.81 ± 0.02**	**6.56 ± 0.02**				
Temperature (°C)	1	37.11 ± 0.03	37.1 ± 0.03	37.08 ± 0.03	37.14 ± 0.05	**37.11 ± 0.3** ^{a}	NS	<0.001	NS
	3	26.68 ± 2.63	29.4 ± 0.26	29.39 ± 0.26	29.29 ± 0.27	**28.69 ± 0.3** ^{b}			
	6	21.07 ± 0.09	21.1 ± 0.18	21.11 ± 0.12	21.11 ± 0.17	**21.1 ± 0.3** ^{c}			
	12	11.74 ± 0.12	11.7 ± 0.1	11.73 ± 0.1	11.70 ± 0.07	**11.72 ± 0.3** ^{d}			
	24	1.6 ± 0.06	1.6 ± 0.06	1.6 ± 0.04	1.59 ± 0.07	**1.60 ± 0.3** ^{e}			
	LS Mean ± SE	**19.64 ± 0.27**	**20.18 ± 0.27**	**20.18 ± 0.27**	**20.17 ± 0.27**				

$^{a, b, c, d, e}$: Different letters in the same column represent statistically significant difference (P<0.05).
$^{A, B, C}$: Different letters in the same column represent statistically significant difference (P<0.05).
NS: Not significant, C: Control (Animals slaughtered after 1 week of lairage).
LT_0: Immediate slaughter (0 lairage time).
LT_{12}: Animals slaughtered after 12 hours of lairage.
LT_{24}: Animals slaughtered after 24 hours of lairage.
G: Group, T: Time, GxT: Group x Time, LS: Least Squares.

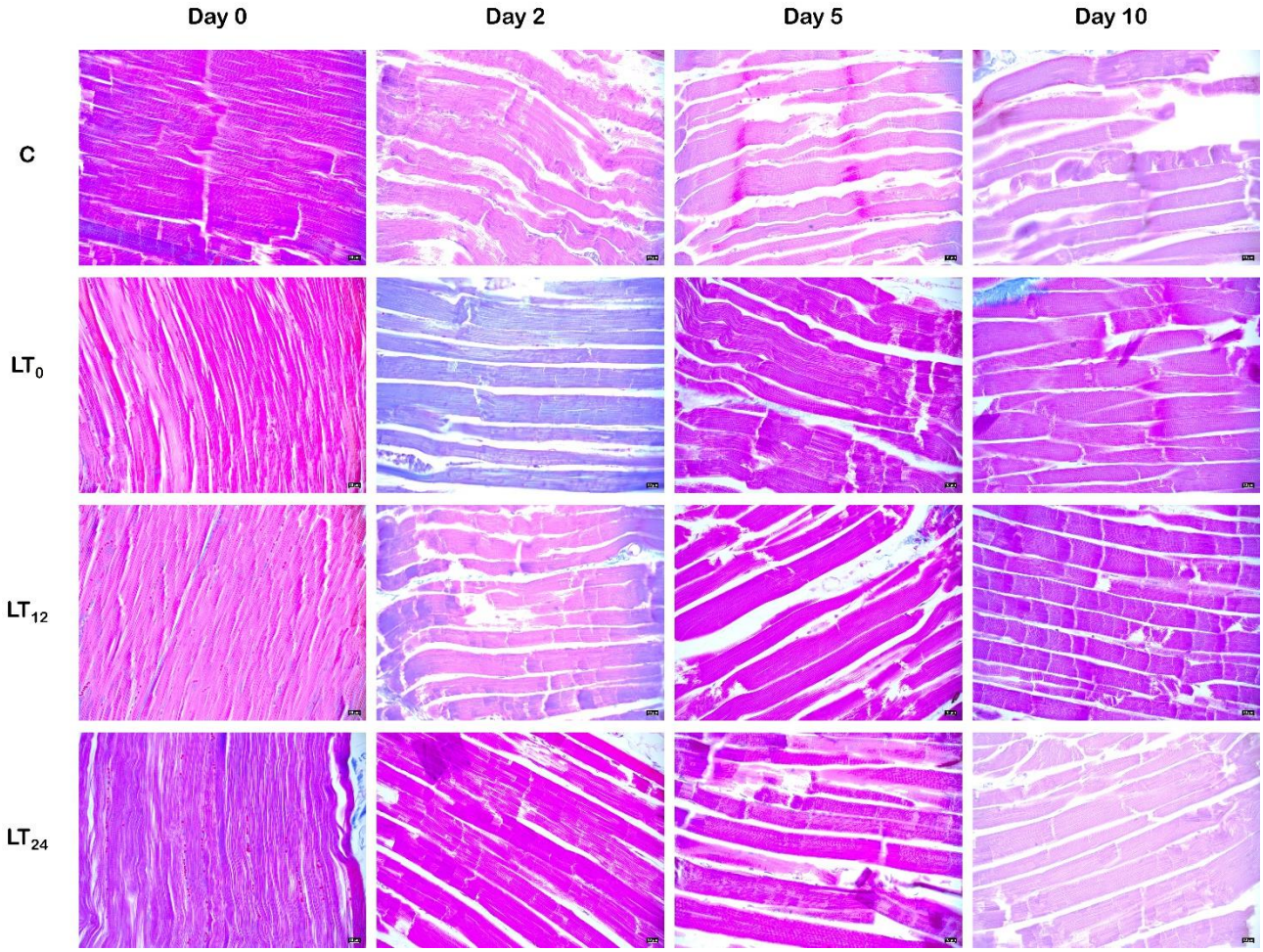

Figure 1. General view of *M. longissimus dorsi* tissue comparing sarcomere lengths. Masson's trichrome staining. Bar: 10 µm.

Conventional light microscopy results were revealed that intercellular space among myofibrils were getting larger towards the day 10 (Figure 1 and 2). Transverse and longitudinal sections of the fibra muscularis were also evaluated with a scanning microscope. According to these results, same as in light microscopy, no change was observed between groups on day 0. In all groups, it was found that the myofibrils homogeneously covered the entire sarcoplasm. On day 2 of maturation, it was noticed that in the cross-sections of the fibra muscularis, intercellular spaces were beginning to be visible among the cells (Figure 3). On day 5, the most remarkable finding was the increase in the number of fat droplets passing outside the cell sarcoplasm (Figure 4). In addition, it was noticed that the myofibrils have lost their homogeneous distribution and started to be separated from each other. As of day 10, it was determined that myofibrils was filled the entire sarcoplasm, and the homogeneous appearance of myofibrils were disappeared due to myofibrillar denaturation and shortening. In addition, the expansion of the intercellular space was confirmed by SEM (Figure 4).

Organoleptic evaluation of the meat samples by the panelists in terms of parameters examined, highest and statistically significant values were obtained for the groups LT_{24} and C (Table 5).

Measurable gene expression was determined for all genes examined in the groups on days 0 and 2nd (< 30Ct). But, due to the rapid degradation of RNA and samples on days 5 and 10, were not included in the molecular analyzes.

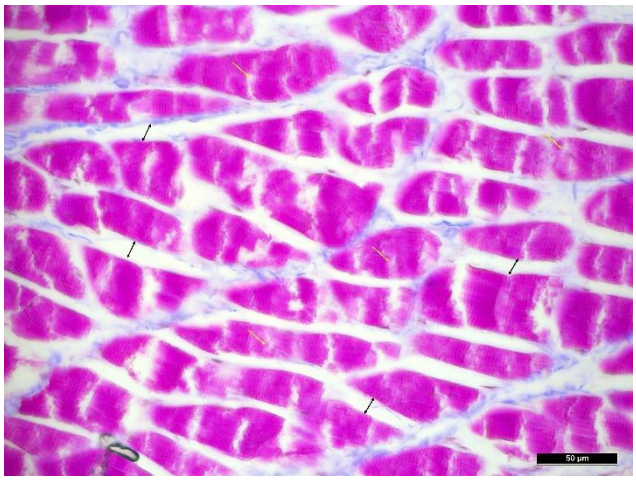

Figure 2. *M. longissimus dorsi* histomorphology on day 10. Increase in intercellular space (double headed arrow), Myofibril denaturation (arrows), Masson's trichrome staining, Bar: 50µm.

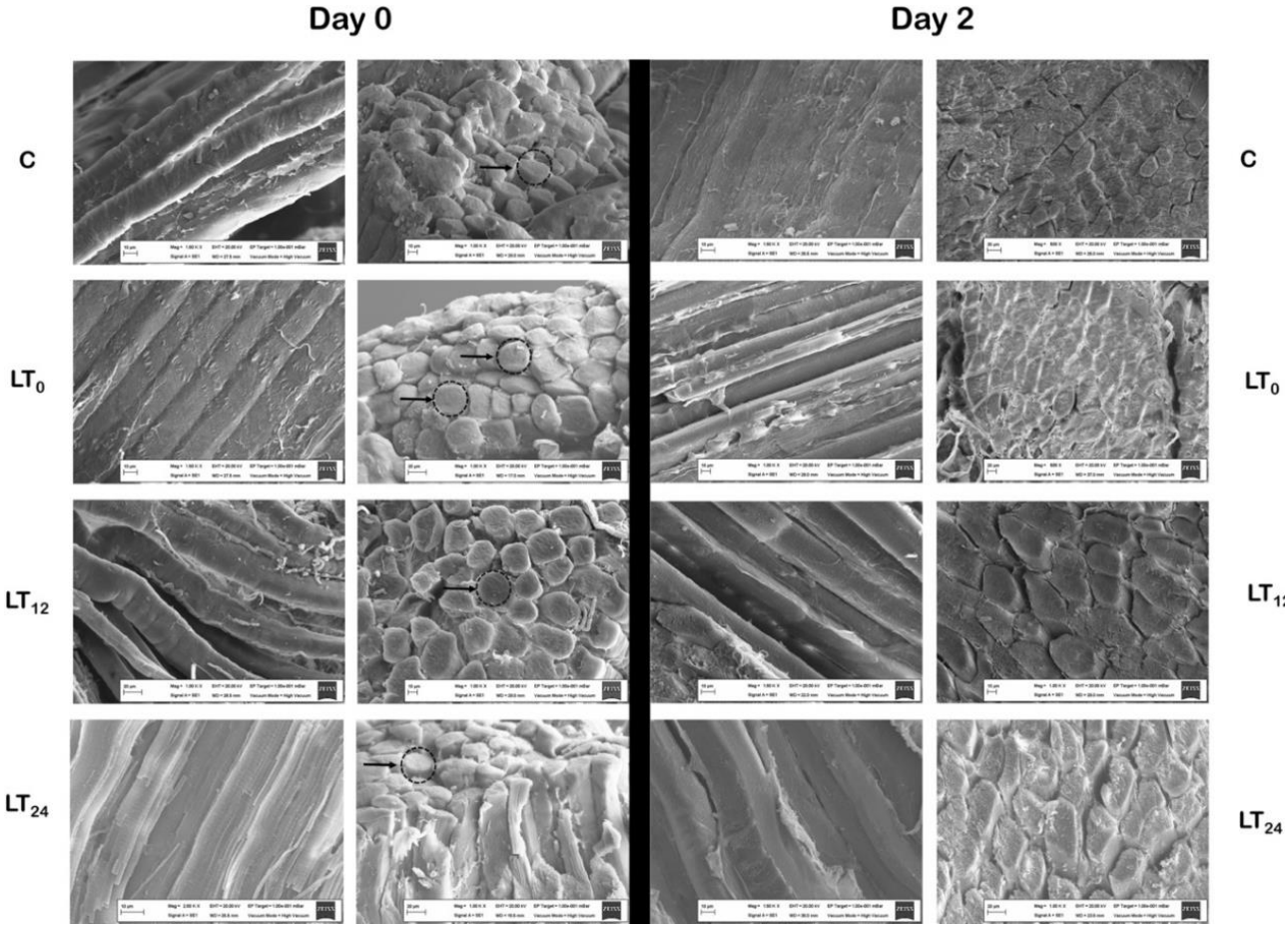

Figure 3. Scanning Electron Microscopy of myofibrils on days 0 and 2. Each circle indicates muscular fibrils, Bar: 10µm.

Table 5. Mean values, standard errors and significant interactions of sensorial characteristics (color intensity, color hue, odor intensity, tenderness, chewiness, juiciness, flavor intensity, flavor quality, and overall acceptability) of sheep meats subjected to short-term transportation and lairage time.

Attribute	Time (day)	C	LT_0	LT_{12}	LT_{24}		Significance		
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	LS Mean ± SE	G	T	G x T
Color intensity	2	5.61 ± 0.08	4.94 ± 0.19	6.7 ± 0.18	6.03 ± 0.12	**5.818 ± 0.11** $^{a\,b}$	**<0.001**	**0.001**	**0.144**
	5	5.58 ± 0.18	5.54 ± 0.36	6.75 ± 0.5	6.54 ± 0.17	**6.104 ± 0.11** ^{a}			
	10	5.4 ± 0.15	5.2 ± 0.06	5.8 ± 0.06	5.67 ± 0.12	**5.517 ± 0.11** ^{b}			
	LS Mean ± SE	**5.53 ± 0.127** ^{B}	**5.226 ± 0.127** ^{B}	**6.416 ± 0.127** ^{A}	**6.079 ± 0.127** ^{A}				
Color hue	2	5.97 ± 0.15	5.33 ± 0.03	6.97 ± 0.32	6.12 ± 0.2	**6.099 ± 0.133**	**<0.001**	**0.153**	**0.467**
	5	5.5 ± 0.24	5.43 ± 0.41	6.46 ± 0.48	6.13 ± 0.2	**5.877 ± 0.133**			
	10	5.13 ± 0.19	4.87 ± 0.19	6.74 ± 0.09	6.2 ± 0.32	**5.736 ± 0.133**			
	LS Mean ± SE	**5.536 ± 0.153** ^{B}	**5.208 ± 0.153** ^{B}	**6.723 ± 0.153** ^{A}	**6.149 ± 0.153** ^{A}				
Odor intensity	2	5.9 ± 0.08	5.76 ± 0.06	5.82 ± 0.05	5.97 ± 0.13	**5.861 ± 0.06** ^{c}	**0.013**	**<0.001**	**0.982**
	5	6.67 ± 0.23	6.46 ± 0.11	6.5 ± 0.13	6.79 ± 0.12	**6.606 ± 0.06** ^{a}			
	10	6.47 ± 0.03	6.13 ± 0.2	6.27 ± 0.08	6.47 ± 0.03	**6.333 ± 0.06** ^{b}			
	LS Mean ± SE	**6.346 ± 0.069** $^{A\,B}$	**6.117 ± 0.069** ^{B}	**6.196 ± 0.069** $^{A\,B}$	**6.408 ± 0.069** ^{A}				
Tenderness	2	7.06 ± 0.29 $^{a\,A}$	6.09 ± 0.23 $^{a\,B}$	6.76 ± 0.23 $^{a\,A}$	6.91 ± 0.07 $^{a\,A}$	**6.705 ± 0.079**	**<0.001**	**<0.001**	**0.016**
	5	6.17 ± 0.25 $^{b\,A}$	5.13 ± 0.03 $^{b\,B}$	5.29 ± 0.02 $^{b\,B}$	6.17 ± 0.06 $^{b\,A}$	**5.688 ± 0.079**			
	10	4.53 ± 0.13 $^{c\,A}$	4.26 ± 0.08 $^{c\,A}$	4.3 ± 0.09 $^{c\,A}$	4.4 ± 0.12 $^{c\,A}$	**4.373 ± 0.079**			
	LS Mean ± SE	**5.921 ± 0.091**	**5.158 ± 0.091**	**5.449 ± 0.091**	**5.826 ± 0.091**				
Chewiness	2	6.39 ± 0.13 $^{a\,A\,B}$	5.94 ± 0.03 $^{a\,B}$	6.76 ± 0.08 $^{a\,A}$	6.61 ± 0.06 $^{a\,A}$	**6.424 ± 0.076**	**<0.001**	**<0.001**	**0.04**
	5	5.25 ± 0.19 $^{b\,A}$	4.17 ± 0.22 $^{b\,B}$	4.88 ± 0.38 $^{b\,A}$	5.38 ± 0.02 $^{b\,A}$	**4.917 ± 0.076**			
	10	5.16 ± 0.05 $^{b\,A}$	4.02 ± 0.06 $^{b\,B}$	4.66 ± 0.09 $^{b\,B}$	5.24 ± 0.07 $^{b\,A}$	**4.77 ± 0.076**			
	LS Mean ± SE	**5.601 ± 0.087**	**4.709 ± 0.087**	**5.431 ± 0.087**	**5.74 ± 0.087**				
Juiciness	2	6.18 ± 0.3	5.55 ± 0.15	6.33 ± 0.04	6.76 ± 0.13	**6.204 ± 0.087** ^{a}	**<0.001**	**<0.001**	**0.701**
	5	5.63 ± 0.38	5 ± 0.06	5.42 ± 0.11	5.75 ± 0.06	**5.448 ± 0.087** ^{b}			
	10	5.53 ± 0.13	4.89 ± 0.03	5.33 ± 0.21	5.62 ± 0.09	**5.346 ± 0.087** ^{b}			
	LS Mean ± SE	**5.781 ± 0.101** ^{A}	**5.146 ± 0.101** ^{B}	**5.693 ± 0.101** ^{A}	**6.044 ± 0.101** ^{A}				
Flavor intensity	2	6.46 ± 0.28	6.08 ± 0.36	6.09 ± 0.23	6.94 ± 0.14	**6.393 ± 0.087** ^{a}	**<0.001**	**0.001**	**0.775**
	5	6.13 ± 0.11	5.64 ± 0.16	6.04 ± 0.12	6.58 ± 0.02	**6.097 ± 0.087** $^{a\,b}$			
	10	6 ± 0.06	5.43 ± 0.04	5.98 ± 0.15	6.31 ± 0.06	**5.931 ± 0.087** ^{b}			
	LS Mean ± SE	**6.194 ± 0.1** ^{B}	**5.718 ± 0.1** ^{C}	**6.038 ± 0.1** $^{B\,C}$	**6.611 ± 0.1** ^{A}				
Flavor quality	2	6.94 ± 0.1	5.94 ± 0.12	6.27 ± 0.07	6.67 ± 0.09	**6.454 ± 0.058** ^{a}	**<0.001**	**0.043**	**0.964**
	5	6.92 ± 0.1	5.96 ± 0.09	6.33 ± 0.21	6.54 ± 0.02	**6.438 ± 0.058** ^{ab}			
	10	6.73 ± 0.1	5.86 ± 0.11	6.12 ± 0.2	6.35 ± 0.04	**6.264 ± 0.058** ^{b}			
	LS Mean ± SE	**6.863 ± 0.067** ^{A}	**5.919 ± 0.067** ^{D}	**6.241 ± 0.067** ^{C}	**6.518 ± 0.067** ^{B}				
Overall acceptability	2	6.24 ± 0.21	5.79 ± 0.15	6.49 ± 0.06	7.06 ± 0.1	**6.393 ± 0.125** ^{a}	**<0.001**	**<0.001**	**0.827**
	5	5.38 ± 0.22	4.54 ± 0.16	5.21 ± 0.2	6.25 ± 0.18	**5.344 ± 0.125** ^{b}			
	10	5.53 ± 0.59	4.47 ± 0.07	5.33 ± 0.12	5.93 ± 0.38	**5.317 ± 0.125** ^{b}			
	LS Mean ± SE	**5.716 ± 0.144** ^{B}	**4.932 ± 0.144** ^{C}	**5.676 ± 0.144** ^{B}	**6.414 ± 0.144** ^{A}				

$^{a, b, c}$: Different letters in the same column represent statistically significant difference (P<0.05).
$^{A, B, C, D}$: Different letters in the same column represent statistically significant difference (P<0.05).
C: Control (Animals slaughtered after 1 week of lairage).
LT_0: immediate slaughter (0 lairage time).
LT_{12}: Animals slaughtered after 12 hours of lairage.

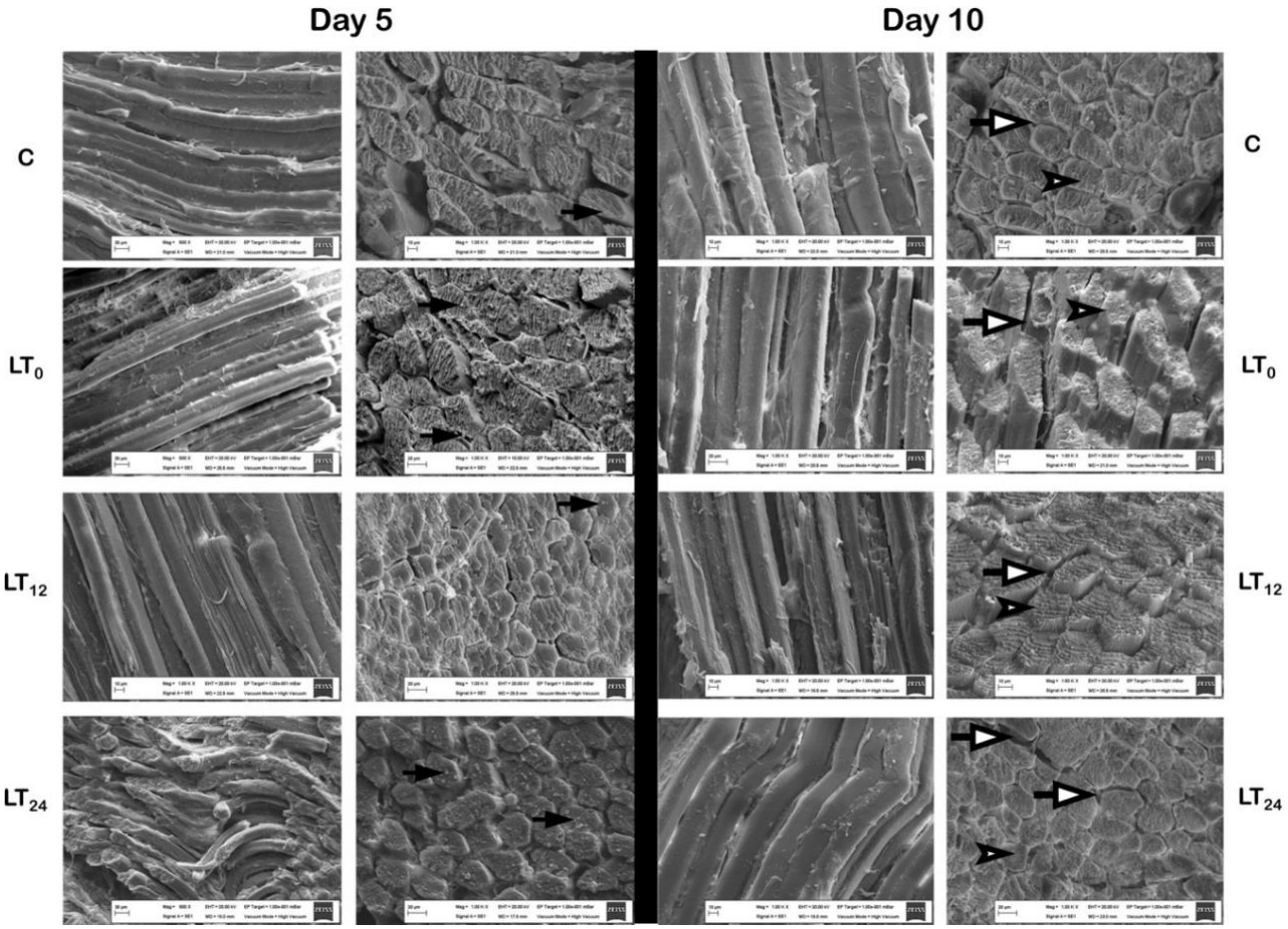

Figure 4. Scanning Electron Microscopy of myofibrils on days 5 and 10. Black arrow: Oil droplets, Arrowhead: Myofibrillar denaturation and myofibrillar shortening, White arrow: Expansion of the intercellular space, Bar: 10µm.

Discussion and Conclusion

The glycogen values difference between the groups were found to be statistically significant (P<0.001). A similar positive effect of lairage time was reported previously by Diaz et al. (12). Also, glycogen values decreased significantly during aging as expected (P<0.001).

The pH values were determined lowest in the longer storage time. These findings were similar to previous publications (18, 28, 35). Significant differences were found between measurements in different control times. The average pH level was different between groups. Similarly, Li et al. (32) and Tomovic et al. (42) reported pH deviations between 5.3-5.7. A recent article has reviewed the pH values and long-term aging positive effects on meat tenderness (38). Cold shortening is described as rapid chilling of the carcass to 10-16°C before pH reaches 6.2 (rigor mortis) (4). In the present study, optimum conditions were determined in L_{24} and C groups (pH 6.2, temperature above 11°C).

The lairage time had no impact on CL and WHC values. No statistically significant difference was found between groups (P>0.05). However, only WHC showed improvement during the aging period, especially on the 10^{th} day, and this was statistically significant (P<0.001). This is validated by earlier studies (7, 11, 30, 40). On the other hand, studies conducted on the effect of lairage time in Kıvırcık lambs revealed significant differences in WHC and CL values (13, 28). In this study, WHC (more water expelled) decreased with storage in all groups. This is due to the increase in drip loss during storage which resulted in free water available for expression.

SF values findings were in correlation with previous studies (13, 26, 36). On the contrary, Ferguson and Warner (14), Ferreira et al. (15), Maria et al. (34) have found no differences between the groups.

Initial color parameters were not affected by PS (P>0.05), however, color values improved in accordance with the aging time (P<0.001) in all groups. Similar to those was found by Kadim et al. (28). However, Ferreira et al. (15) reported that the a* values increased in the groups with high lairage time.

SL values were highest to lowest C, LT_{24}, LT_{12}, and LT_0 groups, respectively. Despite the relatively positive effect of lairage time on the SL, a significant increase in

SL was started with the maturation process of meat (Figure 1). However, the notable alterations at the microscopic level related to the maturation process of the meat started on day 5 and were completed on day 10. In other words, on days 5 and 10, myofibril denaturation and expansion of the intercellular space among the fibra muscularis were significantly ($P<0.001$) increased compared to day 0 (Figure 2).

Although SL and SF showed an inverse ratio, the time x group interaction of these values did not show any significance. A significant relationship, between SF values and SL values, is previously reported (20, 22) and as the SL increases, SF values decrease. Our results were similar to a study conducted on cattle (15), whereas in a sheep study higher SL values of longissimus thoracis muscle were determined in longer lairage time (28). Thus, we speculate that the different species and different muscle types, with additional applications to the expanded lairage time, could have different SF and SL values.

With the increased lairage time (LT_{12}, LT_{24}, and C) the sensation of consistency detected from the product also increased in the direct proportion. Similarly, an increase was observed in the appreciation values perceived by the panelist as a result of increased resting time in flavor intensity and flavor quality. As a result of all these evaluations, panelists determined the general acceptability ranking as LT_{24}> C> LT_{12}> LT_0. Although Ferguson and Warner (14) reported longer transportation time and stress effect on meat tenderness is an indistinct feature, our panelist reported a better sensation in the LT_{24} group than the C group. Similarly, other studies with different lairage time resulted with showed non-significant sensorial findings (36, 48, 49).

The calpain/calpastatin system; *CAPN1*, *CAPN3*, and *CAST* expression measurable for all genes examined in the groups on days 0 and 2nd (< 30Ct). However, on days 5 and 10 expression levels could not be measured. This was thought to be related to the rapid degradation of RNA. Contradictory studies on post-mortem RNA integrity are found in the literature (6, 17). However, studies have also proven that RNA can remain without degradation when preserved under appropriate storage conditions (16). In a study, ante and postmortem samples were compared and a series of transcriptional events that started with the death of the organism were determined. Also, the effects of postmortem gene expression changes of *CAST*, *CAPN1*, and *CAPN3* on meat quality in pigs could be examined (19). In pigs higher *CAST* and *CAPN3* expression was found in correlation with high SF ($P<0.2$) and *CAST* affected the activation time of calpain and drip loss (19). Higher *CAST* expression was associated with poor tenderization ($P=0.0563$) (3). However, in our study RT-PCR results showed that transportation and lairage time did not change expression levels of *CAPN1*, *CAPN3* and *CAST*.

In a study conducted on cattle, higher plasma *HSPA1A* was associated with an in long-time transportation process (8). The transportation duration is one of the most important stress factors for animals. Because the transportation time was set at 3 hours in all groups, the stress marker *HSPA1A* expression didn't show a significant difference between groups. However, *HSPA1A* expression showed significant differences between groups with passing time in the aging process ($P<0.5$). Different lairage times have different effects on *HSPA1A* levels in plasma; in a study, 18 hours lairage time had higher results than 24 hours lairage time (8). In correlation with these findings, a fivefold increase in expression was measured especially in the C and LT_{12} groups. *HSPA1A* protects the cell against destruction by preventing protein degeneration (10) but with our study results, it is not clear whether this increase is due to the deactivation of gene silencing mechanisms associated with cell death or due to the final protection effort of the cell due to the stress caused by cell damage. The high *HSPA1A* plasm level was correlated negatively with SF; positively with pH and meat temperature (8).

The results of this study showed that pH, glycogen, and SF values were found to be lower in groups with higher lairage time (LT_{24}) and control (C). It has been determined that the results obtained with organoleptic evaluations are consistent with the higher effect of lairage time. In contrast, there were no differences between the groups in terms of temperature, CL, color, WHC, and SL values. According to histological evaluations, SL was found to increase starting from day 0 depending on exposure time. However, it was observed that the period of maturation of the meat started with the fifth day. Furthermore, it was determined that the difference between *CAPN1*, *CAPN3*, and *CAST* expressions was not significant, while it was significant for *HSPA1A* expression. Considering that gene expression changes are also affected by variations in the DNA level, it is suggested to design a genotype/phenotype correlation study by larger samples including different strains with additional analyzes, such as determination of genotype, gene expression, and final product proteins, and measurement of enzyme activity at certain intervals.

Financial Support

This work was supported by TÜBİTAK (116O768) and the Research Fund of İstanbul-Cerrahpaşa University (25293).

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

TK, AGB and NB conceived and planned the experiments. TK, AGB, NB, EA, EBB, FGE, AA, FYE and BÇK carried out the experiments. AC, İŞH and AGB planned and carried out the histological analysis. NB, BCK, AGB, TK, AC, DÖ, and ÖC contributed to the interpretation of the results. NB 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

This study was approved by the İstanbul University Animal Experiments Local Ethics Committee (2016/15).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

References

1. **Altınçekiç ŞÖ, Koyuncu, M** (2010): *Nakil koşullarının hayvan refahı üzerine etkileri*. Hay Üret, **51**, 8.
2. **AMSA** (1991): American Meat Association Guidelines for Meat Colour Evaluation. Nat. Live Stock and Meat Board Chicago, USA.
3. **Bagatoli A, Gasparino E, Soares MA, et al** (2013): *Expression of calpastatin and myostatin genes associated with lamb meat quality*. Genet Mol Res, **12**, 4, 6168-6175.
4. **Bendall JR** (1973): *The biochemistry of rigor mortis and cold contracture*. In Proceedings of the 19th European Meeting of Meat Research Workers. Paris, France.
5. **Calvo JH, Iguacel LP, Kirinus JK, et al** (2014): *A new single nucleotide polymorphism in the calpastatin (CAST) gene associated with beef tenderness*. Meat Sci, **96**, 775-782.
6. **Catts VS, Catts SV, Fernandez HR, et al** (2005): *Microarray study of post-mortem mRNA degradation in mouse brain tissue*. Brain Res Mol Brain Res, **138**, 164-177.
7. **Cerdeño AI, Vieira C, Serrano E, et al** (2006): *Carcass and meat quality in Brown fattened young bulls: effect of rearing method and slaughter weight*. J Anim Feed Sci, **15**, 7.
8. **Chulayo AY, Bradley G, Muchenje V** (2016): *Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, HSPA1A and how they relate with meat quality in cattle*. Meat Sci, **117**, 89-96.
9. **Cross HR, West RL, Dutson TR** (1981): *Comparison of methods for measuring sarcomere length in beef semitendinosus muscle*. Meat Sci, **5**, 261-266.
10. **Dangi SS, Gupta M, Nagar V, et al** (2014). *Impact of short-term heat stress on physiological responses and expression profile of HSPs in Barbari goats*. Int J Biometeorol, **58**, 2085-2093.
11. **Del Campo M, Brito G, Soares de Lima J, et al** (2010): *Temperament and lairage time effects on carcass and meat quality traits in steers*. Meat Sci, **86**, 908-914.
12. **Diaz MT, Vieira C, Perez C, et al** (2014): *Effect of lairage time (0h, 3 h, 6 h or 12 h) on glycogen content and meat quality parameters in suckling lambs*. Meat Sci, **96**, 653-660.
13. **Ekiz B, Ekiz EE, Kocak O, et al** (2012): *Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs*. Meat Sci, **90**, 967-976.
14. **Ferguson DM, Warner RD** (2008): *Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants?* Meat Sci, **80**, 12-19.
15. **Ferreira GB, Andrade CL, Costa F, et al** (2006): *Effects of transport time and rest period on the quality of electrically stimulated male cattle carcasses*. Meat Sci, **74**, 459-466.
16. **Ferreira PG, Munoz-Aguirre M, Reverter F, et al** (2018): *The effects of death and post-mortem cold ischemia on human tissue transcriptomes.* Nat Commun, **9**, 490.
17. **Fitzpatrick R, Casey OM, Morris D, et al** (2002): *Postmortem stability of RNA isolated from bovine reproductive tissues*. Biochim Biophys Acta, **1574**, 10-14.
18. **Gallo C, Lizondo G, Knowles TG** (2003): *Effects of journey and lairage time on steers transported to slaughter in Chile*. Vet Rec, **152**, 361-364.
19. **Gandolfi G, Pomponio L, Ertbjerg P, et al** (2011): *Investigation on CAST, CAPN1 and CAPN3 porcine gene polymorphisms and expression in relation to post-mortem calpain activity in muscle and meat quality*. Meat Sci, **88**, 694-700.
20. **Hannula T, Puolanne E** (2004): *The effect of cooling rate on beef tenderness: The significance of pH at 7 degrees C*. Meat Sci, **67**, 403-408.
21. **HNSRKY** (2014): Hayvan Nakilleri Sırasında Refah ve Korunması Yönetmeliği. Available at https://www.resmigazete.gov.tr/eskiler/2014/02/20140205-3.htm Resmi Gazete. (Accessed August 17, 2022).
22. **Holman BWB, Collins D, Kilgannon AK, et al** (2020): *Using shear force, sarcomere length, particle size, collagen content, and protein solubility metrics to predict consumer acceptance of aged beef tenderness*. J Texture Stud, **51**, 559-566.
23. **ISO** (1985): Sensory Analysis – Methodology – General Guidance. International Organization for Standardization, Paris.
24. **ISO** (1988): Sensory Analysis – General Guidance for Design of Test Rooms. International Organization for Standardization, Paris.
25. **ISO** (1993): Sensory Analysis – General Guidance for the Selection, Training and Monitoring of Assessors. Part 1: Selected Assessors. International Organization for Standardization, Genève.
26. **Ivanov NT** (2020): *Sheep welfare during transport and slaughter in Bulgaria – Impact of welfare on slaughter carcass and meat quality: a review*. Turk J Vet Anim Sci, **44**, 174-181.
27. **İnal T** (1995): Kesim Hayvanı ve Et Muayenesi. Saray Kitabevleri, İstanbul.

28. **Kadim IT, Mahgoub O, Al-Marzooqi W, et al** (2009): *Effects of transportation during the hot season and low voltage electrical stimulation on histochemical and meat quality characteristics of sheep longissimus muscle*. Livest Sci, **126**, 154-161.
29. **Kara NK, Koyuncu M** (2011): *Sığırlarda taşıma sırasında hayvan refahına etki eden faktörler*. Kafkas Univ Vet Fak Derg, **17**, 5.
30. **Kerth CR, Braden KW, Cox R, et al** (2007): *Carcass, sensory, fat color, and consumer acceptance characteristics of Angus-cross steers finished on ryegrass (Lolium multiflorum) forage or on a high-concentrate diet.* Meat Sci, **75**, 324-331.
31. **Koohmaraie M** (1996): *Biochemical factors regulating the toughening and tenderization processes of meat*. Meat Sci, **43**S1, 193-201.
32. **Li CB, Chen YJ, Xu XL, et al** (2010): *Effects of low-voltage electrical stimulation and rapid chilling on meat quality characteristics of Chinese Yellow crossbred bulls.* Meat Sci, **72**, 9-17.
33. **Maria GA** (2008). Long Distance Transport and Welfare of Farm Animals. 77-112 In: Meat Quality. CABI, UK.
34. **Maria GA, Villarroel M, Sanudo C, et al** (2003): *Effect of transport time and ageing on aspects of beef quality*. Meat Sci, **65**, 1335-1340.
35. **Mounier L, Dubroeucq H, Andanson S, et al** (2006): *Variations in meat pH of beef bulls in relation to conditions of transfer to slaughter and previous history of the animals*. J Anim Sci, **84**, 1567-1576.
36. **Najafi MH, Mohammadi Y, Najafi A, et al** (2020): *Lairage time effect on carcass traits, meat quality parameters and sensory properties of Mehraban fat-tailed lambs subjected to short distance transportation*. Small Rumin Res, **188**, 106122.
37. **Pfaffl MW** (2001): *A new mathematical model for relative quantification in real-time RT-PCR*. Nucleic Acids Res, **29**, e45.
38. **Pophiwa P, Webb EC, Frylinck L** (2020): *A review of factors affecting goat meat quality and mitigating strategies.* Small Rumin Res, **183**, 106035.
39. **Schaefer AL, Jones SDM, Tong AKW, et al** (1988): *The effects of fasting and transportation on beef cattle. I. Acid-base-electrolyte balance and infrared heat loss of beef cattle*. Livest Prod Sci, **20**, 9.
40. **Teke B, Akdag F, Ekiz B, et al** (2014): *Effects of different lairage times after long distance transportation on carcass and meat quality characteristics of Hungarian Simmental bulls*. Meat Sci, **96**, 224-229.
41. **Terlouw EM, Arnould C, Auperin B, et al** (2008): *Pre-slaughter conditions, animal stress and welfare: current status and possible future research*. Anim, **2**, 1501-1517.
42. **Tomovic VM, Petrovic LS, Dzinic NR** (2008): *Effects of rapid chilling of carcasses and time of deboning on weight loss and technological quality of pork semimembranosus muscle*. Meat Sci, **80**, 1188-1193.
43. **Van de Water G, Verjans F, Geers R** (2003): *The effect of short distance transport under commercial conditions on the physiology of slaughter calves; pH and colour profiles of veal*. Livest Prod Sci, **82**, 8.
44. **Vapnek J, Chapman MS** (2010): Legislative and regulatory options for animal welfare, FAO Legis Study, 104.
45. **Vergara H, Gallego L** (2000): *Effect of electrical stunning on meat quality of lamb*. Meat Sci, **56**, 345-349.
46. **Vergara H, Linares MB, Berruga MI, et al** (2005): *Meat quality in suckling lambs: effect of preslaughter handling*. Meat Sci, **69**, 5.
47. **Vural H, Öztan A** (1996): Et ve Ürünleri Kalite Kontrol Laboratuarı Uygulama Kılavuzu. H Ü Mühendislik Fakültesi Yayınları, Ankara.
48. **Yalcintan H, Akin PD, Ozturk N, et al** (2018): *Effect of lairage time after 2 h transport on stress parameters and meat quality characteristics in Kivircik ewe lambs*. Small Rumin Res, **166**, 41-46.
49. **Yalcintan H, Öztürk N, Akin PD, et al** (2020): *Slaughter and carcass characteristics of Kivircik lambs in different rearing seasons.* Ankara Univ Vet Fak Derg, **68**, 91-95.