Effects of pre-slaughter stress on meat quality characteristics of male lambs of *Hemsin* and *Of* sheep breeds

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1 ABSTRACT

Objective: This study was conducted to determine in meat quality traits in 6-month old sheep breed lambs (Of (n=16) and Hemsin (n=16) which were exposed to pre-slaughter stress (PStr) and not (Cont).
Methods: Half of the lambs (n = 8) from each breed were randomly divided into two groups as pre-slaughter stress (PStr) and control (Cont). Stress was created by huddling together lambs 9 hours before slaughter, which were previously housed in separate compartments not familiar with one another at the end of 60 days fattening period.
Results: Meat pH_{30min} and $pH_{24hours}$ values in *longissimus thoracis et lumborum* (LTL) muscles of hot and cold carcass were influenced (6.22 vs 6.78 and 5.39 vs 5.86) significantly by stress, respectively. There were highly significant correlations between meat pH and quality traits such as cooking loss (R= -0.939, for both pH), water holding capacity (R= -0.924 and -0.892), Warner Bratzler Shear Force (WBSF, R=0.718 and 0.587) for pH_{30min} and $pH_{24hours}$, respectively. Colour brightness values including lightness (L*, 50.28 vs 40.24 and 49.04 vs 40.32), redness (a*, 18.43 vs 16.21 and 19.24 vs16.66) and yellowness (b*, 18.43 vs 16.21 and 7.79 vs 8.76) of LTL muscle standard slices (fresh cut and after 45 minutes) were significantly influenced by pre-slaughter stress treatments. In addition, Chroma values (19.68 vs 18.23 and 20.77 vs 18.85) and hue angles (0.36 vs 0.47 and 0.39 vs 0.49), drip loss (2.37 vs 1.45), cooking loss (25.90 vs27.84), water-holding capacity (WHC, 8.36 vs 7.50), instrumental tenderness (WBSF, 6.62 vs 8.82 kgf/cm^2, P<0.01), hardness (76.08 vs 86.73), adhesiveness (-0.05 vs -0.071) and chewiness (50.72 vs 64.47) were significantly affected by stress. It was concluded based on the present findings that stress caused by bringing together the animals that do not know each other and where a hierarchical social order has not been established influenced the meat quality traits and resulted in qualitative and quantitative losses (1.244 vs 1.725 kg).

2 INTRODUCTION

The increase in the quality of life leads conscious consumer to desire higher quality products in markets. Food is an indispensable need for the survival of all living organisms. Although vegans and vegetarians do not agree, it is generally assumed that animal products should be included into daily diets for a healthy and balanced life. However, changing consumer lifestyles have led the quality traits to become prominent in animal products such as meat. Although the expectations of consumers from meat are similar in almost all regions of the world, there is no consensus on normal meat or quality meat (Monin and Santé-Lhoutellier, 2014). It is inevitable for animals raised for meat production to be exposed to different levels of stress before slaughter. Meat quality obtained from these animals exposed to stress may be negatively affected depending on the severity and duration of the stress (Ferguson and Warner, 2008; Xing *et al.,* 2019). Many factors affect the quality of meat in every phase of meat starting from formation and maturation to consumption. Pre-slaughter stress decreases the meat quality traits through depletion of glycolysis required for the maturation of meat under anaerobic conditions in slaughter (Lowe *et al.,* 2002; Li *et al.,* 2018). Postmortem acidification of muscles is one of the fundamental changes in their conversion into meat. Variation in the rate and extent of the acidification particularly influences meat colour and WHC. The acidification is measured in terms of the pH value of the muscle. Therefore, measuring pH can give valuable information about the potential quality of meat, particularly in situations where more detailed or sophisticated measurements are impossible. The most widely accepted and workable definitions of PSE (Pale-Soft-Exudative) and DFD (Dark-Firm-Dry) meat are in terms of pH values measured at 45th minute and about 24h postmortem (Warriss, 2000). The final pH values of the meat and the decrease in pH in the processes up to the maturation of the meat are under the influence of many factors before and after slaughtering and in particular, the decrease in pH is effective in the appearance and quality parameters of the raw meat (Hughes *et al.,* 2014). Meat colour is an important quality trait and plays a great role in consumer preferences. It is influenced by pre-slaughter stress factors such as exercise, fasting, fear (Zimerman *et al.,* 2013), fighting, transporting(Fisher *et al.,* 2010 , Akin *et al.,* 2018; Liu *et al.,* 2018). Appearance, colour and texture of meat play a greater role in purchase of meat than the other quality traits (Mancini and Hunt, 2005). In Turkey, lamb meat production is carried out by the extensive, semi-intensive or intensive fattening operations. Generally, extensive and semi-intensive growing/fattening conditions are common. At the end of the fattening period, the lambs are transported to the slaughterhouse. It is common for animals that are not from the same herd to come together before slaughter. Such a case causes serious quarrels to create a new social order among the animals brought together until the slaughter. The animals can stay together for a long time until they move to the slaughterhouse. Animals brought to the slaughterhouse from distant or nearby places are not immediately slaughtered because of their resting and sometimes prolonged slaughtering. It is inevitable for animals to meet animals they never knew before or after transport to the slaughterhouse. Animals go through the process until slaughtering at a very high rate as mentioned. This situation leads to significant decreases in meat quality due to the struggle to adapt to a new social environment and to create a new social order in animals. Bringing together animals that do not know each other before or after moving to the slaughterhouse is considered as an important stress factor. The leadership struggle among male individuals in sheep species is a very strong attitude as compared to females (Hargreaves and Hutson, 1997). Therefore, it is estimated that the effect of both leadership struggle and homosexual behaviour on meat quality in males will be greater than the females. While studies about the effects of stress factors including transportation (Li *et al.,* 2018; Liu *et al.,* 2018; Najafi *et al.,* 2020;), heat (Macías-Cruz *et al.,* 2020), exposure to fasting (Zimerman *et al.,* 2013) and thirst (dos Santos *et al.,* 2019) and shearing and washing (Bray *et al.,* 1989; Kannan *et al.,* 2007) and handling (Gregory, 1994; 1996) before slaughter on meat quality have been encountered, no studies have been found on the effects of the fight, which is much more prevalent among domestic breeds, on carcass meat quality. The aim of this study is to investigate the effects of pre-slaughter stress on meat quality traits of the six-month old male lambs that do not know each other and brought together before slaughter. Effects of the struggle of these animals to establish sociological order and the resultant stress on meat quality traits were also investigated.

3 MATERIALS AND METHODS

3.1 Experimental area: The study was conducted in Animal Farm of the Agricultural Research and Application Center (41° N and 36.15° E) of Ondokuz Mayıs University, Samsun, Turkey. All experimental procedures and animal management practices were performed according to the Animal Care and Use Guidelines of Ondokuz Mayıs University of Local Ethical Committee (HADYEK Protocol Number: 2017/25).

3.2 Experimental design and slaughter details: In this study, 32 animals (16 from local breed "Of" and 16 from "Hemsin" breed) were used. The single-born and weaned male lambs with about equal live weights (28.34±0.62 kg) and at the age of about 120 (±6) days were purchased from four private sheep farms at an average distance of 600 km and moved to the fattening area. Sixteen lambs from each of the local sheep breed (*Hemsin* and *Of*) were included in a 60-day individual fattening trial between October - November. The animals were supplied with 450 g roughage and concentrate as much as they could eat during the 60-day diet. Freshwater and mineral block were provided *ad libitum*. Each lamb was housed in individual pans (1.20 m x 1.50 m, 1.8 m^2) to isolate from each other. Roughage and concentrate feed in front of the animals were taken 13 hours before the slaughter. At the end of the first 4 hours of these 13 hours, the animals were weighed and divided into control (n=16) and stress (n=16) groups. The stress group lambs were brought together in a common area of 75 m^2 away from the other group (common, stress-free group) to create stress before slaughter. The stress group (PSrt) was formed in two areas of 37.5 m^2 with two repetitions, 4 animals from each race. At the end of the experiment, since there were no significant differences between the repeats in terms of the parameters considered in the study, the data were combined. The shelter was illuminated by fluorescent lamps and the east side was open, in a position to receive the moonlight. PSrt group animals were kept together for 9 hours from 11:00 pm at night until 8:00 am in the morning. The behaviours of the animals brought together to create stress were observed and recorded for the first hour. The control group (Cont) animals, consisting of 16 lambs (8 male lambs of each breed), were kept in their own compartments until the time of transportation for slaughter. The control group was not affected by the sound and fight of the stress group. After 9 hours of stress conditions, all animals were weighed before being loaded into the transport vehicle for slaughter and this weighing was considered as the live weight at the end of fattening period. Transportation to the slaughterhouse took 15 minutes. All the animals were slaughtered under the "Halal" procedures in a private slaughterhouse at a distance of 5 km from the research farm in the morning at 09.30 am. After carcasses were rested at + 4 ° C for 24 hours, they were divided into two equal parts using an electric saw along the vertebrae by a professional butcher. The *longissimus thoracis et lumborum* (LTL) muscles from the first rib to the last lumbar vertebrae were removed from the left side of the carcasses. Meat colour, drip loss, cooking loss, Warmer Bratzler shear force (WBSF) values were determined (Li *et al.,* 2018). The live weights of the animals were recorded at the beginning of the fattening and at the beginning of the pre-slaughter stress and at the end of the experiment (at the end of the pre-slaughter stress or at the end of the fattening) with a precision scale (±50 g). Immediately after 30 minutes, the slaughter hot carcass weights and after 24 hours + 4 ° C cold carcass weights, pH values of hot and cold carcass and meat colour values were determined.

3.3 Meat pH: The pH was measured manually using a portable pH meter (Cyberscan PC 510) with a penetrating solid-type pH electrode immediately after carcass dressing within 30 minutes and after the carcasses were rested at + 4 ° C for 24 hours from the same location of the *longissimus thoracis et lumborum* (LTL) muscles. An electrode was immersed into the meat and kept until the values were fixed on the display of the pH meter and this fixed value was read and recorded. The measurements were taken from the three different regions of the samples and pH values were found according to the average of these values (Ramirez and Cava, 2007).

3.4 Meat colour: Meat colour (CIE-L*a*b*) was evaluated at 24 h postmortem on a fresh cut surface of LTL muscle using a Colorimeter (Minolta CR-300, Chroma Meter Reflectance) with illuminate C and 11 mm measurement diameter. The Colorimeter was calibrated against a standard white plate and four measurements were made on each sample (Li *et al.,* 2018). The parameters L* (lightness), a* (redness) and b* (yellowness) were recorded. Hue angle was calculated as [arctan (b*/a*)], and the Chroma or saturation index was calculated as $(a^{*2}+b^{*2})^{0.5}$ (Sabbioni *et al.,* 2019; Ye *et al.,* 2020).

3.5 Meat cooking loss and drip loss: Drip loss and cooking loss were measured on the LTL muscles. The first and second lumbar vertebrae were weighed, placed in a plastic bag without contacting with the bag and hung at +4°C for 24 h. The samples were weighed again after gently wiping the sample surface (Honikel, 1998). For determination of the cooking loss of meat, samples of 40-50 g weight were taken from LTL muscles and placed in vacuum bags and cooked in a hot water bath (70 °C) for 40 minutes. Afterward, the samples were kept under the tap water until they cooled down to room temperature (25 ° C) for about 30 minutes and the samples were removed from the bags and reweighed (Honikel, 1998; Mitchaothai *et al.,* 2007). The cooking loss (%) was determined using the weight measurements of the meat samples before and after cooking. To determine the drip loss, 50 g meat samples were taken from the LTL muscles 24 hours after cutting. A net supported the samples taken so that they meet the bag, and they were kept suspended in a plastic container for 48 hours at 2 °C. After this time, the sample taken from the plastic container was weighed again after drying with drying paper. The drip loss of meat was determined as a percentage (%) by proportioning the difference between the first and the last weight measurements of the samples (Bond and Warner, 2007).

3.6 Meat water holding capacity and crude fat percentage: Meat samples taken from the LTL muscles were minced and mixed with (0.6 M) NaCl solution and homogenized by centrifugation at + 4 ° and 10,000 rpm for 15 minutes. The results were recorded as the amount of water absorbed in millilitres per 100 grams of meat (Souza *et al.,* 2016). The percentage of crude meat fat in LTL muscles was determined according to AOAC (2007) official method 960.39.

3.7 *Instrumental tenderness or Warner-Bratzler Shear force (WBSF):* Instrumental tenderness was evaluated by WBSF blade connected to Instron 3343 device. The force applied to the meat in the Instron device was set at 50 kg and the blade speed at 200 mm / min. In texture analysis, samples used in the measurement of cooking loss were used. Samples were taken from these samples in 1 × 1 cm section parallel to the muscle fibres and 3 cm long and were cooked in vacuum bags in a 70 °C water bath for 30 min. Subsequently, they were stored for 3 hours at 4 °C in a refrigerator and the computer recorded the peak shear force (Shear force, kgf / cm2) and force-time graph obtained by measurements. The peak shear force value of the LTL muscle was determined by averaging five measurements (Honikel, 1998; Starkey *et al.,* 2017).

3.8 Statistical analysis: The suitability of all data for parametric tests was checked with Smirnow and Levene tests of SPSS 25.0 software (IBM SPSS, version 25; SPSS Inc., Chicago, IL, USA). Effects of pre-slaughter stress on meat quality traits of the sheep breeds were tested with generalize linear model (GLM) of SPSS software. Breeds and treatments (exposed to stress, PStr and not released, Cont) were taken as fixed factors. All data are presented in mean ± standard error of the mean (SEM). The differences between the factors were evaluated with the t-test. Relationships between meat quality parameters were determined by Pearson correlation, and for this purpose Cont and PStr applications were considered as two different sets (n=16).

4 RESULTS AND DISCUSSION

4.1 Effects on live weight and carcass traits: It was determined that a 9 hours of stress created by bringing together animals that do not know each other did not significantly influence the final body weights. However, body weight loss in animals exposed to stress was about half a kilo more than in the control group. Similarly, effects of pre-slaughter stress on hot and cold carcass weights and carcass yield were not significant for both breeds (Table 1 and Figure1).

Table 1: Live weights at the beginning and end of fattening and hot and cold carcass weights in animals exposed to stress before slaughter.

Parameters	Appl	Of	Hemsin	P level¹	Overall Mean	P level²
Body weight at beginning (kg)	Cont	28.42±1.035	28.72±1.136		28.57±0.713	0.867
	PStr	28.85±1.412	27.87±1.365		28.36±0.928	
	GM	28.64±0.814	28.29±0.837	0.642		
Body weight at stress (kg)	Cont	41.93±1.643	40.39±2.924		41.16±1.580	0.890
	PStr	42.25±1.544	40.61±1.691		41.43±1.104	
	GM	42.09±1.045	40.50±1.564	0.263		
Body weight at finishing (kg)	Cont	40.59±1.304	39.24±2.876		39.91±1.484	0.906
	PStr	40.74±1.144	38.68±1.218		39.71±0.866	
	GM	40.66±0.804	38.96±1.449	0.624		
Hot carcass weight	Cont	20.25±0.723	19.35±1.295		19.80±1.000	0.780
	PStr	19.38±0.661	19.73±0.816		19.55±0.491	
	GM	19.81±0.483	19.44±0.712	0.585		
Cold carcass weight (kg)	Cont	20.00±0.636	19.03±1.213		19.51±0.660	0.622
	PStr	19.13±0.554	1948±0827		19.30±0.466	
	GM	19.56±0,707	19.25±0.685	0.624		
Hot dressing percentage (%)	Cont	49.85±1.965	49.66±1.114		49.76±1.057	0.077
	PStr	45.83±1.769	47.85±1.691		46.84±1.051	
	GM	47.84±1.324	48.75±0.998	0.557		
Cold dressing percentage (%)	Cont	48.95±1.773	48.95±1.072		48.95±0.803	0.091
	PStr	45.51±1.249	46.99±1.862		46.25±1.074	
	GM	47.23±1.196	47.97±1.061	0.628		
Crude fat (%)	Cont	1.388±0.059	1.555±0.042		1.471±0.060	0.05
	PStr	1.213±0.035	1.468±0.056		1.340±0.067	
	GM	1.300±0.040	1.511±0.036	0.01		
Loin eye area (cm^2)	Cont	20.72±0.526	20.59±0.553		20.66±0.369	0.01
	PStr	17.67±0.696	16.53±1.352		17.10±0.752	
	GM	19.20±0.577	18.56±0.886	0.585		

Appl: application; GM: general mean; 1: inter-breed p-values; 2: inter-treatment p-values

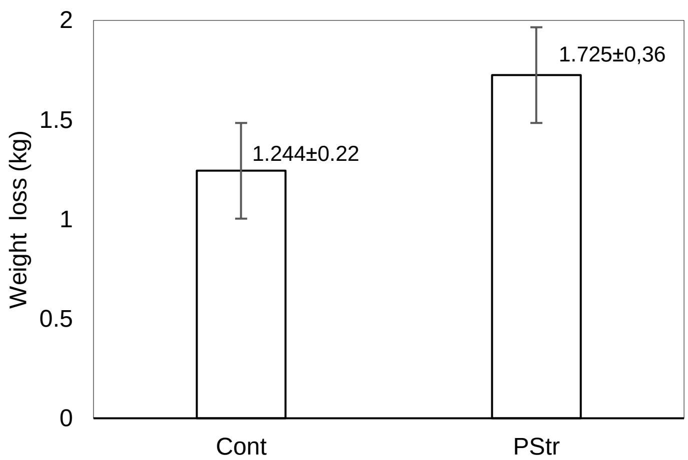

Figure 1: Live weight (kg) losses in animals until the preparation time for slaughter, whether they are exposed to stress (PStr) before slaughter or not (Cont).

In practice, when it was considered that the animals (especially, aggressive, horned and harsh temperament of some Turkish local sheep breeds including these two) were bought and brought together from different herds, this stress period lasts more than 9 hours. In these conditions, the values of the properties, as mentioned earlier, might be affected negatively. It was observed that the fibres covering the bodies of some animals in the stress group were excessively moist and there were physical injuries in the carcasses after slaughter. In practice, it is also said that there were serious injuries and even deaths caused by quarrels between the animals. Although it is said that the most common stress factor before the slaughter was due to transport (Eriksen *et al.,* 2013), it was believe based on the present findings that the most effective stress factor on meat quality was the bringing together of animals that do not know each other before the slaughter and the stress caused by social order or unknown fights between these animals. It was observed that bringing together male lambs who did not know each other started a great fight and continued a complete confusion with the participation of all the animals into the group. In the later hours of the fight, it was observed that some animals participated into the fight by beating one side to the other in favour of those who struggled, and even by making homosexual jumps that mounting like in times of mating. The struggle of other lambs against the lamb, which tends to establish superiority, prevented the formation of a social hierarchy. For this reason, it was not easy for an animal to become dominant. It was determined that the live weight losses of the animals in the preparation stage for slaughter were approximately half a kilogram more in the stress group as compared to the control group. If too many animals from different flocks are brought together, serious injuries and deaths might occur, as well as the fight will take longer and weight loss and meat quality will be adversely affected. The power spent in this struggle was reflected as more weight loss in the animals of the stress group (Table 1 and Figure 1). It was determined that the fat accumulation between LTL muscle fibres of Hemsin breed male lambs were higher ($P < 0.01$) than Of breed male lambs. It is accepted that breed difference was an important factor in the accumulation of fat between muscle fibres. The difference in crude fat percentages between the treatment and the control groups may be resulted from the energy required during the fight from the body fat reserves. Even after slaughter, body fat reserves might have been used for the needs of stretched muscle tissues due to excess stress (England *et al.,* 2017). Loin eye area values were significantly lower in the stress group animals than in the control group animals. Stress caused by pre-slaughter fighting had a worse effect than leaving them without water for 72 hours (Dos Santos *et al.,* 2019). There was no effect of breed and treatment on the organic matter, crude protein, dry matter and crude ash contents of meat (data were not shown).

4.2 Meat pH values: Effects of breeds and experimental treatments on pH of the lamb meat were provided in Table 2. The pH value of meat, affected by several factors such as handling, fighting, transportation and exercise, which animals are exposed to before slaughter, has important effects on meat quality traits such as drip loss, water holding capacity, colour and protein functionality (Li *et al.,* 2018). In this study, the differences in pH values of hot and cold carcasses of the animals exposed to stress before slaughter were found to be significant, while the effects of the breeds were not found to be significant. It might be said that the stress caused by the fight made to establish social order among male lambs before slaughter was greater than the stress caused by hunger, exercise and fear (because physical fight involves all stress conditions at the highest rate) (Zimerman *et al.,* 2013). In the studies on meat pH values, there is no consensus about the effect of the breeds on meat pH. While the present findings were not compatible with those who reported that the breed was effective on pH (Jandasek *et al.,* 2014; Hajji *et al.,* 2016; Stewart *et al.,* 2018), they were compatible with those who stated that the breed was not effective on meat pH (Teixeira *et al.,* 2005; Abdullah *et al.,* 2011). It was seen that the pH changes of hot and cold carcass of each breed were very close to each other. The similarity of pH values in Hemsin and Of breeds can be attributed to the likelihood of their glycolytic potential being the same (Abdullah *et al.,* 2011).

Table 2: The pH of hot and cold carcass and the values of cooking and drip loss in animals exposed to stress before slaughter.

Breeds	Treatments	pH hot carcass	pH cold carcass	Cooking loss	Drip loss	WHC
Of	Cont	6.20±0.031	5.38±0.051	24.62±0.435	2.404±0.096	8.46±0.07
	PStr	6.78±0.036	5.85±0.046	26.98±1.078	1.496±0.083	7.56±0.11
	GM	6.49±0.111	5.62±0.094	25.80±0.639**	1.950±0,181	8.01±0.18
Hemsin	Cont	6.24±0.033	5.39±0.039	27.17±0.164	2.342±0.079	8.36±0.09
	PStr	6.78±0.028	5.86±0.041	28.70±0.602	1.410±0.051	7.50±0.11
	GM	6.49±0.105	5.63±0.092	27.93±0.360**	1.876±0.181	7.93±0.17
Cont		6.22±0.022**	5.39±0.030**	25.90±0.398*	2.373±0.059**	8.36±0.06**
PStr		6.78±0.021**	5.86±0.029**	27.84±0.613*	1.453±0.048**	7.50±0.08**

*: P<0.05; **: P<0.01; WHC: water holding capacity

In several studies examining the effects of the stress factors on meat quality, the pH value of the hot carcass (Li *et al.,* 2018) and both hot and cold carcasses were reported to be unaffected (Zimerman *et al.,* 2013), but Li *et al.*(2018) reported that pH values in LTL muscles of cold carcass were affected by transportation stress. The decrease in pH values of hot and cold carcasses in the stress group can be attributed to significant glycogen consumption levels in the muscles in the stress group (Gregory, 1994; 1996). In this study, the decrease in pH during the aging of meat was greater than desired (Jandasek et al, 2014; Honikel, 2014). Effects of pre-slaughter stress on meat pH were found to be similar with the findings of Macias-Cruz *et al.*(2020) reporting the effects of heat stress on meat pH of wool-type lambs and with the findings of Najafi *et al.*(2020) reporting the effects of transportation stress on meat pH of Mehreban fat-tailed lambs. The correlations between meat pH values and meat quality traits are provided in Table 3. Such correlations revealed important information for comprehension of the factors affecting meat pH before slaughter to reach the desired quality in

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meat. The meat of the "Of" lambs, which have lower fat content among the muscle fibres, had greater drip loss than the "Hemsin" lambs (Cheng and Sun, 2008). In the PStr group, drip loss was found much higher than the Cont group.

Table 3: Pairwise Pearson Correlations (Corr.) Between pH and Meat Parameters (MP)

P-Value	95% CI for ϱ	Corr.	pH1	MP	pH2	Corr.	95% CI for ϱ	P-Value
0.000	(0.828; 0.979)	0.938	pH1	pH2	pH2			
0.171	(-0.165; 0.726)	0.360	pH1	Cook Loss	pH2	0.361	(-0.165; 0.726)	0.170
0.000	(-0.979; -0.828)	-0.939	pH1	Drip Loss	pH2	-0.939	(-0.979; -0.829)	0.000
0.000	(-0.974;-0.791)	-0.924	pH1	WHC	pH2	-0.897	(-0.964;- 0.723)	0.000
0.000	(-0.947; -0.615)	-0.851	pH1	L*1	pH2	-0.806	(-0.930; -0.516)	0.000
0.005	(-0.875; -0.259)	-0.669	pH1	a*1	pH2	-0.737	(-0.903; -0.381)	0.001
0.021	(0.102; 0.831)	0.569	pH1	b*1	pH2	0.599	(0.147; 0.844)	0.014
0.000	(-0.917; -0.449)	-0.773	pH1	L*2	pH2	-0.669	(-0.875; -0.260)	0.005
0.011	(-0.851; -0.170)	-0.614	pH1	a*2	pH2	-0.759	(-0.912; -0.422)	0.001
0.057	(-0.015; 0.790)	0.484	pH1	b*2	pH2	0.474	(-0.028; 0.785)	0.063
0.002	(0.345; 0.895)	0.718	pH1	WBSF	pH2	0.587	(0.128; 0.838)	0.017
0.006	(0.233; 0.868)	0.653	pH1	Hardness	pH2	0.566	(0.098; 0.829)	0.022
0.014	(-0.843; -0.145)	-0.598	pH1	Adhesive	pH2	-0.494	(-0.795; 0.003)	0.052
0.040	(0.030; 0.807)	0.518	pH1	Chewiness	pH2	0.523	(0.037; 0.809)	0.038
0.002	(0.344; 0.895)	0.717	pH1	Hue1	pH2	0.767	(0.437; 0.915)	0.001
0.006	(0.231; 0.867)	0.652	pH1	Hue2	pH2	0.725	(0.359; 0.898)	0.001
0.032	(-0.816; -0.057)	-0.537	pH1	Chroma1	pH2	-0.601	(-0.845; -0.150)	0.014
0.029	(-0.819; -0.067)	-0.544	pH1	Chroma2	pH2	-0.702	(-0.888; -0.316)	0.002

CI: Correlation interval, WBSF: Warner Bratzler shear force; WHC: water holding capacity

4.3 Meat cooking loss: Effects of both the breeds and stress treatments on cooking loss were found to be significant (Table 2). Meat cooking loss consists of water-soluble proteins and fats of the meat, so cooking losses in the meat are required to be low (Miller, 2014). Abdullah *et al.,* (2011) reported that effects of the genotypes on cooking loss of meat were significant for *semitendinosus* muscle, not for LTL muscle. There are different findings about the effects of breeds on cooking loss of meat. Martinez-Cerezo *et al.*(2005), Abdullah *et al.*(2011), Burke *et al.*(2003), Cheng and Sun, 2008) stated that the effects of breed or genotype on meat cooking loss were significant, while Abdullah *et al.*(2011, in LTL muscles), Hajji *et al.*(2016) indicated that breed was not effective on cooking loss of meat. The effect of breed on cooking loss could be due to the difference in its ability to bind collagen to muscle fibres (Cheng and Sun, 2008). In may be thought that breeds' ability to bind collagen to muscle fibres may be similar in genetically close breeds. Different types of stress including temperature (Liu *et al.,* 2016), transport (Li et al, 2018; Najafi *et al.,* 2020) and fasting (Karaca *et al.,* 2016) were reported to effect the cooking losses of meat. While the hot carcass, cold carcass pH values and the cooking loss and drip loss of the meat are not affected by breeds, it was determined that the aforementioned values of the animals exposed and not exposed to stress were affected.

4.4 Water holding capacity: Water holding capacity is an important quality criterion because it affects the colour, sensory quality and acceptable appearance of the meat (Cheng & Sun, 2008; Keeton *et al.,* 2014). The PStr group had a negative effect ($P < 0.01$) on the water holding capacity of the LTL muscles in animals as compared to the Cont group. The results obtained are not compatible with the findings of Zimerman et al (2013) indicating that stress (starvation, exercise and fear) did not affect meat WHC. This mismatch could be attributed to the difference in stress tolerance levels of Hemsin and Of sheep breeds. The timid attitude of the Hemsin breed when the lambs feed has been observed to be very high. As stated before, research involving contradictory results about the effects of breed and pre-slaughter stress on meat water holding capacity could be found. These differences may vary depending on the level of perception of stress by breeds and their stress tolerance levels. While there was a positive and tend to be a significant relationship between pH values and cooking loss in this study, a negative and significant relationship was found between pH values and drip loss and between pH values and water holding capacity (Table 2 and Table 3). It was stated that as meat pH reaches the isoelectric point, meat loses more water as drip loss during the storage or water loss during the cooking. The greater the drip and cooking loss is, the drier and tougher the meat is. Therefore, meat pH is an important component of meat quality related to the ability of muscle proteins to bind water and the subsequent juiciness and tenderness of the meat (Miller, 2014). Water holding capacity, which is influenced by various internal and external factors, defined as the ability of the meat to retain water both inherent and additional water is an important quality criterion. However, it is reported that pH played a key role in water holding capacity of the meat (Cheng and Sun., 2008).

4.5 Warner Bratzler Shear force (WBSF) or instrumental tenderness: Mean instrumental tenderness (WBF) values obtained from the LTL muscles in the PStr group animal carcass were significantly higher than those of the Cont group (Table 4). Tenderness of meat is an important quality criterion. Since the tenderness assessment by the panellist or consumers is subjective, an objective method based on the response to the shear force applied to a standard piece of meat has been developed (Hopkins, 2014).

Table 4: Meat Shear force power (WBSF), hardness, stickiness, chewiness and resilience values in animals exposed to stress before slaughter or not.

Breed	Appl	WBSF(kgf/cm^2)	Hardness	Adhesiveness	Chewiness	Resilience
Of	Cont	6.16±0.655	71.98±3.654	-0.045±0.015	45.98±4.400	0.287±0.027
	PStr	8.64±0.453	85.41±4.192	-0.071±0.004	65.02±9.066	0.221±0.008
	GM	7.40±0.596	78.70±3.616	-0.058±0.009	55.50±5.891	0.249±0.017
Hemsin	Cont	7.09±0.682	80.88±2.345	-0.054±0.003	55.46±7.372	0.238±0.007
	PStr	8.99±0.172	88.05±2.503	-0.071±0.005	64.32±5.542	0.236±0.036
	GM	8.04±0.486	84.12±2.174	-0.062±0.004	59.89±4.586	0.237±0.017
Cont		6.62±0.472**	76.08±2.539**	-0.050±0.007**	50.72±4.358*	0.257±0.015
PStr		8.82±0.234**	86.73±2.314**	-0.071±0.003**	64.67±4.920*	0.229±0.017

**: P<0.01,*: P=0.05

As the instrumental tenderness value increases, the chewing and crushing of the meat require more power, so the instrumental tenderness value should be low in terms of meat quality. The findings obtained in this study were similar to the shear force values that were obtained from animals exposed to transportation stress (Abdullah *et al.,* 2011; Ekiz *et al.,* 2012; Eriksen *et al.,* 2013; Akin *et al.,* 2018; Macias-Cruz *et al.,* 2020) and were not similar with those of (Abdullah *et al.,* 2011; Zimerman *et al.,* 2013). In general, WBSF values were found to be higher than the values found by Zimerman *et al.,* 2013; Abdullah *et al.,* 2011; Akin *et al.,* 2018; Macias-Cruz *et al.,* 2020). These differences might be due to age (Polidori *et al.,* 2017; Ye *et al.,* 2020) or breed differences of the animals (Teixeria *et al.,* 2005; Abdullah *et al.,* 2011, Xing *et al.,* 2019), or the difference of the method used (Fabre at al., 2018), and/or to the very active living conditions along with the herd at the high altitudes of the animals before two months of feeding. Instrumental tenderness values were reported to vary depending on the maturation process of the meat, collagen content and soluble collagen (Starkey *et al.,* 2017). Stress may have influenced collagen-related factors acting on instrumental tenderness. It was determined that the hardness, adhesiveness and chewiness characteristics, which were the quality characteristics of the meat, were significantly affected by stress. However, it was determined that the quality characteristics of springiness, cohesiveness, gumminess and resilience were not affected by neither breed nor treatment.

4.6 Meat colour: Meat colour, one of the most critical criteria that consumers consider when purchasing meat (Mancini and Hunt, 2005, Hajji *et al.,* 2016), was significantly negatively affected by stress in this study (Table 5 and Table 6). The main difference in meat colour was thought to be due to ultimate pH (pH_{24} in this study) differences (Ye *et al.,* 2020; Bekhit *et al.,* 2019). However, in studies on the effects of different stress factors on meat colour, various results were encountered that support (Macías-Cruz *et al.,* 2020) and do not support (Zimerman *et al.,* 2013; Bond and Warner, 2007) present findings. The most logical explanation for these contradictory results may be that different breed (Xing *et al.,* 2019) animals had different levels of tolerance to stress.

Table 5: L*(lightness), a* (redness), b* (yellowness) colour values in cold carcass new steaks and 45 minutes later in animals exposed to stress before slaughter or not.

Breed	Appl	Cold carcass (0 min)			Cold carcass (45 min)		
		L*1	a*1	b*1	L*2	a*2	b*2
Of	Cont	50.57±1.155	18.87±0.823	7.15±0.211	52.94±1.547	19.58±0.807	8.18±0.222
	PStr	40.22±2.476	16.28±0.743	8.45±0.297	39.97±2.188	15.88±0.828	8.75±0.339
	GM	45.40±2.328	17.58±0.710	7.80±0.298	46.46±2.247	17.73±0.880	8.46±0.217
Hemsin	Cont	50.18±2.001	17.99±0.709	6.60±0.361	45.14±1.625	18.89±0.608	7.40±0.406
	PStr	40.25±1.865	16.14±0.189	8.08±0.821	40.67±1.435	17.44±0.892	8.77±0.566
	GM	45.22±2.265	17.07±0.488	7.34±0.500	42.90±1.312	18.17±0.570	8.08±0.413
Cont		50.38±1.072**	18.43±0.530**	6.88±0.220**	49.04±1.804**	19.24±0.485**	7.79±0.260*
PStr		40.24±1.435**	16.21±0.356**	8.27±0.410**	40.32±1.218**	16.66±0.635**	8.76±0.305*

*: P<0.05; **: P<0.01

Table 6: Hue angle and Chroma indices in the steaks of LTL muscles from cold carcass in animals exposed to stress before slaughter or not.

		Cold carcass (0 min)		Cold Carcass (45 min)	
Breed	Appl	Hue 1	Chroma1	Hue2	Chroma2
Of	Cont	0.363±0.025	20.20±0.704	0.398±0.016	21.23±0.780
	PStr	0.478±0.016	18.35±0.752	0.505±0.019	18.15±0.822
	GM	0.422±0.025	19.27±0.591	0451±0.023	19.69±0.784
Hemsin	Cont	0.353±0.019	19.18±0.693	0.375±0.027	20.31±0.506
	PStr	0.463±0.043	18.10±0.249	0.465±0.041	19.57±0.698
	GM	0.407±0.308	18.64±0.397	0.421±0.029	19.94±0.424
Cont		0.359±0.7147**	19.68±0.496**	0.386±0.014*	20.77±0.464*
PStr		0.471±0.0220**	18.23±0.370**	0.487±0.022*	18.85±0.567*

*: P<0.05, **: P<0.01, 1.

It has been reported by consumers that the gloss value should not be lower than 34 in order to accept the edible meat (Hajji *et al.,* 2016). In the study, although the stress decreased the lightness (L*) value of the meat as compared to the control group, its acceptability in terms of colour was not influenced by stress. Negative effects of stress before slaughter on the decrease of the final pH and on the water holding capacity caused the colour of the meat to be negatively affected (Bekhit *et al.,* 2019). The effect of stress on the Chroma index, which is the measurement value of colour intensity (Abdullah *et al.,* 2011), was significant in both fresh meat cuts and meat cuts measured after 45 minutes. Similarly, the effect of stress on Hue angle values was also significant (Table 6). Significant correlations were found between meat quality parameters and pH values and between meat quality parameters (Table 3 and Table 7).

Table 7: Pairwise Pearson correlations (Corr.) between some meat quality parameters (MP)

Parameter 1	Parameter 2	**Correlations**	**95% CI for P**	**P-Value**
WBSF	Cooking loss	0.487	(-0.011; 0.792)	0.056
WBSF	Drip loss	-0.663	(-0.872; -0.249)	0.005
WBSF	WHC	-0.674	(-0.877; -0.268)	0.004
WBSF	L*1	-0.593	(-0.842; -0.138)	0.015
WBSF	a*1	-0.575	(-0.833; -0.111)	0.020
WBSF	b*1	0.490	(-0.008; 0.793)	0.054
WBSF	L*2	-0.663	(-0.872; -0.250)	0.005
WBSF	Hardness	0.654	(0.234; 0.868)	0.006
WBSF	Adhesiveness	-0.553	(-0.823; -0.079)	0.026
WHC	L1*	0.857	(0.628; 0.949)	0.000
WHC	L*2	0.784	(0.472; 0.922)	0.000
WHC	a*1	0.726	(0.360; 0.898)	0.001
WHC	a*2	0.600	(0.148; 0.844)	0.014
WHC	Cooking loss	-0.380	(-0.737; 0.142)	0.146
WHC	Drip loss	0.970	(0.914; 0.990)	0.000
WHC	Loin area	0.561	(0.091; 0.827)	0.024
WHC	Hardness	-0.561	(-0.827; -0.091)	0.024
WHC	Adhesiveness	0.494	(-0.003; 0.795)	0.052
WHC	Chewiness	-0.588	(-0.839; -0.130)	0.017
Adhesiveness	Hardness	-0.526	(-0.810; -0.041	0.036
Springiness	Hardness	0.493	(-0.004; 0.795)	0.052
Chewiness	Hardness	0.578	(0.116; 0.835)	0.019
Chewiness	Springiness	0.680	(0.278; 0.879)	0.004
Resilience	Gumminess	-0.548	(-0.821;-0.072)	0.028
Drip loss	Loin area	0.630	(0.195; 0.858)	0.009
Adhesiveness	Loin area	0.553	(0.079; 0.823)	0.026
Cohesiveness	Loin area	-0.830	(-0.939; -0.568)	0.000
Hardness	Drip loss	-0.562	(-0.827; -0.091)	0.024
Renk L*1	Drip loss	0.812	(0.529; 0.932)	0.000
Renk a*1	Drip loss	0.696	(0.307; 0.886)	0.003
Renk L*2	Drip loss	0.739	(0.384; 0.904)	0.001
Renk a*2	Drip loss	0.672	(0.264; 0.876)	0.004
Adhesiveness	Drip loss	0.492	(-0.006; 0.794)	0.053
Chewiness	Drip loss	-0.566	(-0.829; -0.098)	0.022

CI: Correlation interval, WBSF: Warner Bratzler shear force; WHC: water holding capacity

Negative, strong and reliable relationships were determined between pH values and drip water loss, WHC, colour values L*1, L*2, a*1, a*2. It has been determined that there are positive, strong and reliable relationships between pH values and WBSF, hardness, b*1 colour values. Similar results were reported by Okeudo and Moss (2008). Based on the presence of strong relationships between pH values and the other meat parameters, it is misleading to state that this strong relationship caused strong relationships between the other quality parameters (Hassler and Thadewald, 2003), but it will not eliminate the possibility of such a relationship. Therefore, the relationship of meat quality parameters with each other shows that each can create a domino effect in terms of meat quality. For this reason, a great care must be taken in all phases of meat from production to consumption.

5 CONCLUSION

This study reveals the effects of stress on the quality of meat and loss of live weight by bringing together animals that do not know each other before slaughter. The stress caused by the fight made to create social order among the animals negatively affected the meat quality characteristics such as water holding capacity, drip loss, final pH value, cooking loss, colour values (lightness, redness, yellowness, chroma, and hue), instrumental tenderness, hardness, adhesiveness and chewiness.

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