EFFECTS OF STOCKING DENSITY OF LAMBS ON BIOCHEMICAL STRESS PARAMETERS AND MEAT QUALITY RELATED TO COMMERCIAL TRANSPORTATION*

Bulent Teke^{1*}, Bulent Ekiz², Filiz Akdag¹, Mustafa Ugurlu¹, Gulay Ciftci³,
Berrin Senturk⁴

¹Department of Animal Breeding and Husbandry, Ondokuz Mayis University, Veterinary Faculty,
55200 Atakum, Samsun, Turkey

²Department of Animal Breeding and Husbandry, Istanbul University, Veterinary Faculty,
34320 Avcilar, Istanbul, Turkey

³Department of Biochemistry, Ondokuz Mayis University, Veterinary Faculty,
55200 Atakum, Samsun, Turkey

⁴Department of Animal Health Economics and Management, Ondokuz Mayis University,
Veterinary Faculty, 55200 Atakum, Samsun, Turkey

*Corresponding author: bulentteke@gmail.com

Abstract

The aim of this study was to evaluate the effects of stocking density (0.20 and 0.27 m²/lamb) during transportation on certain biochemical stress parameters and meat quality characteristics of fifty-five Karayaka lambs divided into two groups. The front compartment of the trailer was loaded at high stocking density (HD: 0.20 m²/lamb; n=33) and the remainder was loaded at low stocking density (LD: 0.27 m²/lamb; n=22). The distance was approximately 130 km and duration was 2 h 15 min. Blood samples were taken just before and just after transportation. Glucose (P<0.001), lactate (P<0.001), cortisol (P<0.01), creatine kinase (CK) (P<0.01), lactate dehydrogenase (LDH) (P<0.01) and alanine aminotransferase (ALT) (P<0.05) levels were significantly higher in lambs of the HD group than in the LD group. The effects of stocking density on pH, proportion of expressed juice, cooking loss, Warner-Bratzler shear force value and meat colour parameters were not significant (P>0.05). Transportation with a stocking density of 0.20 m²/lamb resulted in higher stress responses in lambs compared with a stocking density of 0.27 m²/lamb. However, increased stress in the HD group did not lead to any adverse effects on meat quality characteristics. In the determination of stocking density, a compromise that gives animal welfare and commercial objectives equal status would improve the current situation.

Key words: lamb, meat quality, road transportation, stocking density, stress parameters

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Transportation of animals involves various stressful procedures, such as herding on the farm, loading, travel, noise, vibration, physical discomfort and unloading. These stressors may cause changes in certain biochemical parameters such as the plasma concentrations of glucose, lactate, cortisol, CK and LDH, which are used to assess the level of stress in sheep (Tadich et al., 2009; Miranda-de la Lama et al., 2011; Ekiz et al., 2012 a; Piccione et al., 2013). Most farm animals are transported at least once during their lifetime, including farm to farm or to a slaughterhouse (Zhong et al., 2011) and the adverse effects of transportation on animal welfare can be minimized by appropriate management (Fisher et al., 2009).

Besides its adverse effects on animal welfare, stress experienced by farm animals during transportation may also cause economic losses due to decreased carcass and meat quality (Giannetto et al., 2011; Teke et al., 2014). In the case of increased physiological stress or physical activity during transportation, muscle glycogen reserves may be used before slaughter which can lead to higher ultimate meat pH, darker meat colour, tougher meat and greater water holding capacity (Gregory, 1998).

The level of stress in sheep experienced during transport is influenced by many factors, such as transport duration (Fisher et al., 2010; Dalmau et al., 2013), stocking density (Cockram et al., 1996; Fisher et al., 2002, 2010), social mixing (Ekiz et al., 2013), logistic stopovers (Miranda-de la Lama et al., 2010, 2012 a), driving style (Fisher et al., 2005) and road conditions (Cockram et al., 2004; Miranda-de la Lama et al., 2011).

The effects of stocking density on animal welfare are one of the most important aspects of animal transportation (Hall and Bradshaw, 1998). For example, if animals cannot move and lie down comfortably because of high stocking density, the welfare of the animal can be adversely affected (Knowles et al., 1995). In Council Regulation (EC) No 1/2005 of the European Union regarding the protection of animals during transport and related operations, a minimum space of 0.20 to 0.30 m²/animal must be provided for shorn sheep or lambs weighing less than 55 kg, depending on breed, size, physical condition, environmental conditions and journey time. To elicit further information on the impacts of transportation on lamb, the objective of the present study was to compare certain stress parameters and meat quality characteristics of Karayaka lambs transported at stocking densities of 0.20 and 0.27 m²/lamb, which almost concur with the lower and upper limits of EU regulations.

Material and methods

The research protocol of the current study was approved by the Ethics Committee of Ondokuz Mayıs University in Samsun, Turkey (Approval number: 2010/68). The city of Samsun is located in the central Black Sea region of northern Turkey and has a typical Black Sea climate. Rainfall is distributed throughout the year and average annual precipitation is 800 mm. Summers are warm and humid, with average annual relative humidity of 76%. The average maximum temperature is 27°C in August. Winters are cool and humid, and the lowest average minimum temperature is 3°C in January.

Animals and treatments

A total of 55 clinically healthy Karayaka lambs (approximately 90 days old and 29.0 ± 1.4 kg live weight) were used. They were randomly divided into two groups (HD: High density group, and LD: Low density group) 12 h before road transportation. All animals were individually numbered on their flank with green (LD) or red (HD) livestock spray paint. Lambs had free access to water and dry clover until transportation.

The livestock transporter (Isuzu, NPR) used in the study was adequately suited for sheep, with two axles and one floor. The dimensions of the trailer were 5.45 m length $\times$ 2.30 m width $\times$ 1.25 m height. The front compartment of the trailer was used for lambs of the HD ($0.20 \text{ m}^2/\text{lamb}$; $n = 33$) group and the rear was used for the LD ($0.27 \text{ m}^2/\text{lamb}$; $n = 22$) group. Space allowances for both groups were within the range of values recommended by the European Commission (2006).

The lambs were transported from the Vezirköprü district (latitude $41^\circ 19' \text{ N}$, longitude $35^\circ 01' \text{ E}$ and 380 m above sea level) to a public slaughterhouse (Samsun Municipality Slaughterhouse) (latitude $41^\circ 27' \text{ N}$, longitude $36^\circ 34' \text{ E}$ and 5 m above sea level) in Samsun city, Turkey. The journey of approximately 130 km from farm to slaughterhouse started at 08:00 a.m. and took 2 h 15 min. Transportation was during summer and the ambient temperature ranged from 24°C to 27°C , and the average humidity was 68%. The temperature-humidity index (THI) was calculated from the temperature and humidity data supplied from the device (YCOM KMN-303), according to the formula used by West (1994). THI values were between 71 and 76 during the journey which was completed during mild heat stress conditions, in terms of the THI heat-stress thresholds, as defined by Silanikove (2000). Average speed of the lorry was 58 km/h and the journey was completed without stops and sudden acceleration and deceleration. The project personnel observed loading, transportation and unloading to ensure compliance with regulations and experimental procedures. Loading of the lambs was carried out as described by Ekiz et al. (2012 b), but unloading was done via a ramp which was suitably angled.

Blood sampling and biochemical analyses

Prior to transportation, the lambs were weighed carefully to minimize stress. Two blood samples were collected from each lamb's jugular vein into vacutainer tubes containing anticoagulant (EDTA); the first sample was taken just before transportation and the second sample was collected just after transportation. The same trained person collected all the blood samples throughout the study, and sampling was completed in <1 min per animal to minimize handling stress that may have affected the results.

The collected blood samples were kept on ice for two hours before delivery to the laboratory. The samples, which were in EDTA coated tubes, were centrifuged at $1500 \times g$ for 10 min. The plasma fractions were separated and immediately frozen at -25°C until the day of analysis. Plasma glucose, CK, LDH, ALT and lactate levels were analysed with diagnostic kits (Auditdiagnostic, Cork, Ireland). Plasma concentrations of cortisol were measured with a diagnostic ELISA direct immunoenzymatic kit (DiaMetra, Foligno, Italy).

Meat quality analyses

Lambs were slaughtered at the slaughterhouse by severing the major blood vessels in the neck 60 min after unloading. The pH of the *longissimus thoracis* muscle was measured between the 12th and 13th thoracic vertebrae immediately after dressing (pH₀), after 45 min (pH₄₅), and at 24 h post slaughter (pH_{ult}), with a digital pH meter (Testo 205, Testo Inc., Sparta, NJ). The *longissimus thoracis* muscle from the left side of the carcass was excised at 24 h postmortem for assessment of water holding capacity (cooking loss and expressed juice), the Warner-Bratzler shear force (WBSF) value, and meat colour parameters. Meat quality was analysed after the aging of the *longissimus thoracis* muscle at 4°C for 7 days. To measure the percentage of expressed juice, a modified Grau and Hamm method, as described by Beriain et al. (2000), was used.

Meat samples taken from the *longissimus thoracis* muscle between the 5th and 11th thoracic vertebrae were used for determination of cooking loss percentage. Samples were packed under vacuum and cooked for 45 min in an 80°C water bath (Honikel, 1998). After the measurement of cooking loss, the cooked samples were used for shear force measurement. The procedure described by Ekiz et al. (2012 b) was adopted to prepare samples for shear force analysis. Briefly, six sub-samples, cut parallel to the muscle fibres and with a cross section of 1×1 cm and 2.5–3 cm length, were excised from the cooked meat samples. A Warner Bratzler shear force apparatus connected to an Instron Universal Testing Machine (Model 3343, Instron Corp., Norwood, MA, USA) was used for shear force measurements at a crosshead speed of 150 mm/min.

A Minolta chromometer (Model CR 400, Minolta Camera Co., Osaka, Japan) was used to determine meat colour, and the standards reported by the CIE (1976) were applied. Light source, aperture size and observation angle were set at D65, 8 mm and 2°, respectively. A standard white plate (Y=93.8, x=0.316, y=0.3323) was used for calibration of the chromometer. Colour measurements were performed immediately after cutting, on the cut surface of 2.5 cm thick samples from the fat-free area, on the 1st and 7th days of storage. Meat samples were kept at 4°C and under continuous white light (750 lx) during the 7-day storage period. The chroma (C*) and hue angle (H*) were calculated with the following formulas, as previously reported by Wyszecki and Stiles (1982):

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2}; H^* = \tan^{-1} (b^*/a^*)$$

Statistical analyses

The repeated measurement of ANOVA in the SPSS 10.0 statistical package (SPSS, 1999) was used to analyse biochemical stress parameters. The model included sampling time as a within-subject effect and stocking density group as a between-subject effect. The independent samples T-test was used for the statistical analyses of meat quality data. Least squares means are reported throughout, and probability values of $P < 0.05$ were considered to be statistically significant.

Results

Biochemical parameters

The effects of stocking densities of 0.20 and 0.27 m²/lamb on biochemical stress parameters in blood samples taken immediately after transport were significantly different (Table 1). Plasma concentrations of glucose and lactate after transport were significantly higher in lambs of the HD group than in lambs of the LD group ($P<0.001$). CK, LDH and ALT concentrations measured after transportation were also significantly higher in lambs of the HD group than in lambs of the LD group ($P<0.01$; $P<0.01$ and $P<0.05$, respectively).

Table 1. Means and standard errors (SE) for certain biochemical parameters in high and low stocking density groups

Characteristics	High Density (HD)		Low Density (LD)		Sig. ^d
	mean	SE	mean	SE	
Glucose (mg/dl)					
initial	60.91	3.66	64.43	4.28	ns
final	69.73 a	4.17	46.49 b	4.57	***
Lactate (mg/ml)					
initial	23.10	0.56	22.80	0.62	ns
final	25.40 a	0.56	31.26 b	0.30	***
Cortisol (ng/ml)					
initial	18.42	6.57	16.65	2.30	ns
final	49.24 a	3.21	29.90 b	2.84	**
CK (U/l)					
initial	170.70	23.01	197.41	18.28	ns
final	191.24 a	15.66	144.32 b	14.36	**
LDH (U/l)					
initial	112.82	2.62	115.52	4.42	ns
final	172.21 a	3.39	150.94 b	3.81	**
ALT (U/l)					
initial	18.55	1.01	16.67	4.78	ns
final	29.91 a	3.41	20.57 b	0.22	*

HD – (0.20 m²/lamb); LD – (0.27 m²/lamb).

d – significance level of differences between HD and LD groups.

a, b – means in the same line with different superscripts are significantly different.

ns – $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Meat quality characteristics

The effects of stocking density on meat quality characteristics are presented in Tables 2 and 3. There was no significant effect of stocking density on meat pH₀, pH₄₅ and pH_{ult} ($P>0.05$). The effects of stocking density on proportion of expressed juice

and cooking loss were also not significant ($P>0.05$). In addition, the stocking density did not influence the meat colour characteristics investigated in the present study, except for the a^* and C^* values measured immediately after excision of the samples.

Table 2. Means and standard errors (SE) for pH, WHC, cooking loss and Warner Bratzler Shear Force (WBSF) values of *longissimus dorsi* muscle in high and low stocking density groups

Characteristics	High Density (HD)		Low Density (LD)		Sig.
	mean	SE	mean	SE	
pH ₀	6.34	0.08	6.26	0.05	ns
pH ₄₅	6.01	0.07	6.09	0.05	ns
pH _{ult}	5.55	0.01	5.56	0.01	ns
WHC (%)	10.61	0.50	11.55	0.59	ns
Cooking loss (%)	28.17	0.44	28.32	0.57	ns
WBSF (kg)	4.19	0.41	4.51	0.31	ns

HD – (0.20 m²/lamb); LD – (0.27 m²/lamb).

ns – $P>0.05$.

Table 3. Means and standard errors (SE) for meat colour characteristics of *longissimus dorsi* muscle in high and low stocking density groups

Characteristics	High Density (HD)		Low Density (LD)		Sig.
	mean	SE	mean	SE	
Colour parameters at 0 h					
(L*) ^{0 h}	38.01	0.67	38.22	0.61	ns
(a*) ^{0 h}	17.49 a	0.14	16.58 b	0.85	**
(b*) ^{0 h}	1.69	0.29	1.35	0.32	ns
(C*) ^{0 h}	17.60 a	0.14	16.68 b	0.22	**
(H*) ^{0 h}	5.50	0.96	4.63	1.08	ns
Colour parameters at 1 d					
(L*) ^{1 d}	37.55	0.57	39.01	0.48	ns
(a*) ^{1 d}	16.69	0.30	16.08	0.55	ns
(b*) ^{1 d}	4.96	0.34	4.67	0.46	ns
(C*) ^{1 d}	17.43	0.37	16.79	0.65	ns
(H*) ^{1 d}	16.45	0.84	15.81	0.95	ns
Colour parameters at 7 d					
(L*) ^{7 d}	35.66	1.08	35.51	0.30	ns
(a*) ^{7 d}	15.26	0.81	14.30	0.49	ns
(b*) ^{7 d}	4.00	0.68	3.29	0.30	ns
(C*) ^{7 d}	15.90	0.84	14.72	0.49	ns
(H*) ^{7 d}	13.98	2.62	13.01	1.15	ns

HD – (0.20 m²/lamb); LD – (0.27 m²/lamb).

a, b – means in the same line with different superscripts are significantly different.

ns – $P>0.05$; ** $P<0.01$.

Discussion

Biochemical parameters

Muscle glycogen levels may fall and lactate levels may increase due to a sudden increase in stress or physical exercise, since muscle glycogen is consumed by gluconeogenesis (Apple et al., 1995).

Glucose and lactate concentrations are used as indicators of stress (Tarrant et al., 1992; Miranda-de la Lama et al., 2010, 2011; Zhong et al., 2011). Lactate and glucose concentrations in lambs of the HD group increased in the current study, probably due to the extra energy needed to adapt to higher stocking density. Miranda-de la Lama et al. (2011) found that lambs transported on unpaved roads had significantly higher plasma glucose levels than those transported on paved roads. Tarrant et al. (1992) reported elevated plasma glucose levels associated with increasing stocking density in cattle. The results obtained in the current study were in accordance with that report and also that of Miranda-de la Lama et al. (2011).

Cortisol concentration after transportation was significantly higher in lambs of the HD group than in the LD group. Plasma cortisol concentrations have been reported as a reliable indicator of stress level in lambs (Fordham et al., 1989; Ekiz et al., 2012 a). According to Ali et al. (2006), Ekiz et al. (2012 a, 2013), the increased plasma cortisol concentration due to transport stress might be due to the stimulation of the hypothalamo-pituitary-adrenal axis. Increased plasma cortisol and glucose levels detected in the present study after transportation and handling were consistent with the increases observed in previous studies reported for sheep, goats and cattle (Tarrant et al., 1988; Ruiz-de la Torre et al., 2001; Kannan et al., 2003; Miranda-de la Lama et al., 2011; Ekiz et al., 2012 a, 2013; Ergul-Ekiz and Yalcintan, 2013). Further corroboration of the current result comes from Ibanez et al. (2002) who also reported that lambs of a high stocking density group ($0.125 \text{ m}^2/\text{lamb}$) had higher cortisol concentrations than lambs of a low stocking density group ($0.25 \text{ m}^2/\text{lamb}$).

To be able to stand or maintain the body position in a moving vehicle is physically difficult and exhausting for an animal (Miranda-de la Lama et al., 2011, 2012 b). The CK enzyme is released into the blood when the animal has muscular damage, becomes fatigued, or during vigorous exercise (Knowles, 1998, 1999; Liu et al., 2008; Ekiz et al., 2012 a). LDH and ALT activity are also associated with stress and muscular fatigue (Broom, 2000; Ekiz et al., 2013). Therefore, these enzymes have been used as indicators of trauma, vigorous exercise and tissue damage related to transportation and pre-slaughter stress (Bórnez et al., 2009; De la Fuente et al., 2010; Ergul-Ekiz and Yalcintan, 2013). Miranda-de la Lama et al. (2012 b) reported a correlation between aggressive interactions and plasma CK activity. Ekiz et al. (2013) reported increased CK and LDH levels due to transportation stress in rams and ewes. Tarrant et al. (1992) reported higher plasma cortisol and CK level in steers transported at a high stocking density ($585 \text{ kg}/\text{m}^2$) than those transported at stocking densities of 448 and $500 \text{ kg}/\text{m}^2$. The results obtained in the current study were in accordance with the results of the two previous studies. In contrast, Cockram et al. (1996) did not detect any significant differences among stocking densities (0.22, 0.27, 0.31 and $0.41 \text{ m}^2/\text{lamb}$) in terms of the plasma cortisol concentration and plasma CK activity

of weaned lambs after 12 h transportation. Similarly, De la Fuente et al. (2010) also reported that the effects of stocking density (0.12, 0.20 and 0.25 m²/lamb) on plasma CK, LDH and cortisol levels were not significant.

Meat quality characteristics

There was no significant effect of stocking density on meat pH₀, pH₄₅ and pH_{ult} values; pH_{ult} was below 6.0, which indicates acceptable meat quality for commercial lambs (Gregory, 2007). Pre-slaughter handling may affect glycogen storage and muscle physiology, which affect the ultimate pH (Gregory, 1998). Ultimate meat pH levels, which were within the acceptable range in the current study, indicate that stocking densities of 0.20 and 0.27 m²/lamb do not result in excessive depletion of glycogen. Kannan et al. (2003) stated that the effect of transport stress (130 km) on meat pH_{ult} of young and old goats was not significant. De la Fuente et al. (2010) determined that lambs transported by road to the slaughterhouse at three different stocking densities (0.12, 0.20 or 0.25 m²/lamb) had pH_{ult} values that were not significantly different (5.47, 5.60 and 5.53, respectively). Dalmau et al. (2013) stated that the effect of transport duration (1 h and 24 h) on the pH_{ult} of lamb meat was not significant at normal stocking density. The pH results of the current study are consistent with those reports. Ekiz et al. (2012 b) stated that the proportion of expressed juice and cooking loss are closely related to the ultimate pH of meat. De la Fuente et al. (2010) stated that proteins bind more strongly with water at higher muscle pH values, which allows less free water. Apple et al. (1995) found that meat from stressed lambs had higher ultimate pH and lower cooking loss than from control lambs. Kannan et al. (2003) stated that the effect of transport stress on water holding capacity was not significant. Corroboration of the results of the present study comes from De la Fuente et al. (2010) who reported that the effects of stocking density on the proportions of expressed juice and cooking loss were not significant. The effect of stocking density on the WBSF value was not significant ($P>0.05$) in the current study. Similarly, De la Fuente et al. (2010) also detected no significant effect of stocking density on the WBSF value.

Meat colour is one of the most important indicators of meat quality and freshness for the consumer (Faustman and Cassens, 1990). According to Knowles et al. (1999) and Dalmau et al. (2013), meat from stressed lambs was darker than that from non-stressed lambs. Stress factors such as stocking density and transport duration are the main reasons for dark-cutting meat in sheep (Warriss et al., 1990). However, dark cutting meat was not observed in the present study. This indicates that the transportation stress experienced by Karayaka lambs at stocking densities of 0.20 and 0.27 m²/lamb during a relatively short transport period does not lead to dark-cutting meat. Support for the results of the current study comes from De la Fuente et al. (2010) and Kannan et al. (2003) who stated that the effects of stocking density on meat colour parameters were not significant.

Conclusions

The results of the biochemical stress parameter determinations in the present study clearly showed that transportation with a stocking density of 0.20 m²/lamb

resulted in higher stress responses in lambs when compared with a stocking density of 0.27 m²/lamb. Therefore, 0.27 m²/lamb is a more appropriate allocation in commercial transportation when animal welfare is the primary consideration. On the other hand, lambs transported with stocking densities of 0.20 and 0.27 m²/lamb had similar meat quality characteristics, so the former allocation could be recommended when priority is given to transport costs and meat quality. Giving equal priority to animal welfare and commercial outcomes when determining stocking density for transportation appears to be a socially responsible compromise.

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