Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
http://dx.doi.org/10.5424/sjar/2013112-3274

Spanish Journal of Agricultural Research 2013 11(2), 394-404
ISSN: 1695-971-X
eISSN: 2171-9292

Effect of season and stocking density during transport on carcass and meat quality of suckling lambs

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Abstract

Many factors related to transport to abattoir affects meat quality, but scarce information is available in suckling lambs. Thus, the aim of this work was to evaluate the effects of season and stocking density on carcass and meat quality of suckling lambs during commercial transport to the abattoir. A factorial design (2×3) was used: two seasons (winter and summer) and three stocking densities (SD; 0.08, 0.12 and 0.20 m^2 animal^{-1}). Meat quality variables were measured in the *M. longissimus* at 24 h *post-mortem* and after 5 days of refrigerated storage. Lambs transported in summer showed lower liver weight ($p < 0.001$), h* ($p < 0.05$), deoxymyoglobin content ($p < 0.001$), pressed juice ($p < 0.01$), shear force ($p < 0.001$) and firmness ($p < 0.001$), and higher initial pH ($p < 0.001$), L*, b*, C* ($p < 0.001$) and a* ($p < 0.01$), as well as metmyoglobin and oxymyoglobin content ($p < 0.001$), than those transported in winter. The effect of season was dependent on storage time, being colour changes more evident at 24 h than after 5 days of storage, whereas lipid oxidation was only observed in stored meat, which may be explain because the natural antioxidative system decreases with time after slaughter. Scarce effect of SD was found on the carcass and meat quality parameters, thus under our experimental conditions the three SD studied appear to be suitable for suckling lambs transport. However, both carcass and meat quality were within the normal commercial range.

Additional key words: ageing; colour; light lambs; season; space allowance; texture.

Introduction

Suckling lamb meat is a traditional and typical product of the Mediterranean countries, considered by consumers as a high quality food because of its low fat levels, pale-pink colour, mild flavour and tenderness (Sañudo *et al*., 1997), with an increasing demand during the Christmas and Easter. Suckling lambs are based on dairy sheep breeds, fed exclusively with milk and slaughtered after a suckling period of 30-35 days when have reached 10-12 kg live weight (LW) on average (De Rancourt *et al*., 2006).

Transport to slaughterhouse is one of the final steps in meat production. Many factors related to transport can affect carcass and meat quality and, among them, stocking density (SD) should be taken into account. Thus, high SD have been found to be very detrimental to sheep welfare (Warriss *et al*., 2003) and have been associated with higher mortality rates than low SD, whereas higher bruise scores are often found at low SD because of the increased possibility of the animals falling down (Grandin & Gallo, 2003). On the other hand, environmental conditions (temperature and humidity) during transport can affect to the animals (Kadim *et*

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Received: 27-06-12. Accepted: 09-05-13.

Abreviations used: LW (live weight); MDA (malonaldehyde); SD (stocking density); SD1 (stocking density: 0.08 m^2 animal^{-1}); SD2 (stocking density: 0.12 m^2 animal^{-1}); SD3 (stocking density: 0.20 m^2 animal^{-1}); TBARS (thiobarbituric acid-reactive substance); THI (temperature-humidity index); WHC (water holding capacity).

al., 2004; 2008). In this respect, in Spain, high temperatures (more than 40°C) can be registered in summer, with humidity below 40%, while in winter the temperature may not reach 5°C and the humidity is about 60%, therefore the potential effect of environmental conditions during transport on carcass and meat quality could be different between season (De la Fuente *et al*., 2004). Taking these considerations into account, an appropriate space allowance during transport should be evaluated on the basis of breed, age, physical dimensions, LW, presence of wool, etc. (Knowles *et al*., 1998) as well as environmental conditions. In this respect, Richardson (2002) recommends reducing the SD for sheep transport at high temperatures. Previous authors (Dalla Costa *et al*., 2007; Kadim *et al*., 2008; De la Fuente *et al*., 2010) have studied the transport-based factors, such as SD and season, on welfare and carcass and meat quality in different species. However, little information about these aspects is available on suckling lambs.

Current animal transport regulations are strict for most animals but are rather vague as regards suckling animals. Thus, the Farm Animal Welfare Council (FAWC, 1991; OJ, 2005) suggest specific maximum areas based on animal weight, although the minimum area is not indicated and other aspects are not taken into account. This lack of regulation gives great freedom to operators to transport a large number of animals. As far as season is concerned, Council Regulation (EC) 1/2005 (OJ, 2005) states that the kind and number of animals, as well as the meteorological conditions expected during transport, should be taken into account, but does not specify temperature limits for journeys of less than 8 h. Therefore SD during transport of animals should be adjusted depending on the season in order to minimize the impact on welfare and on carcass and meat quality. In view of the lack of agreement among researchers and the limited information about the effect of transport conditions on carcass and meat quality of young animals, a study of these parameters is clearly necessary. The aim of this study was to evaluate the effects of season and SD on carcass and meat quality of suckling lambs during commercial transport to the abattoir.

Material and methods

The experiment was performed in early March (winter transport, with an environmental temperature of between 7.3 and 11.7°C and humidity of between 53.5% and 87.9%) and in late June (summer transport, with environmental temperatures of between 14.0 and 26.1°C and humidity of between 66.3% and 82.1%), in northwest Spain (41º 30' N and 5º 45' W) at an altitude between 656 and 740 m asl. This area is characterised by a continental climate, with an average annual temperature of 12.7°C and average annual rainfall of 363 mm. Seasons are very marked with hot summers (maximum: 29.6°C; minimum: 11.8°C) and cold winters (maximum: 11.4°C; minimum: 0.6°C), with wide thermal amplitudes.

Experimental design

A total of 72 male suckling lambs Lacaune breed were used for this experiment. Animals with a LW of between 12 and 14 kg (13.06 ± 0.16 kg; suckling lamb commercial category) were selected from an intensive milk farm. They were raised exclusively on natural milk and remained with their mothers until loading. The ewes were milked once a day during lamb breeding. Ewes were fed with alfalfa hay and the same commercial concentrate (1:1) in both seasons. The commercial concentrate diet (g kg^{-1}) was based on maize 700, soybean meal 160, wheat middling 120, calcium phosphate 15, common salt 3, mineral and vitamins premix 2. The mineral and vitamin premix contained (per kg): 150 g Ca, 100 g P, 100 g Na, 100 mg Co, 300 mg I, 5,000 mg Fe, 10,000 mg Mn, 20,000 mg Zn, 100 mg Se, 5,000,000 IU retinol, 500,000 IU cholecalciferol and 15,000 mg α-tocopherol.

The lambs were transported from the farm to the abattoir by road, for 3 h, at three different stocking densities (SD1-0.08 m^2 lamb^{-1}, SD2-0.12 m^2 lamb^{-1} and SD3-0.20 m^2 lamb^{-1}; 6 lambs per SD) according to specifications from the FAWC and Council Regulation (EC) 1/2005. Two replicates (*i.e.,* journeys from the farm to the abattoir) were performed over two consecutive weeks for each season (18 lambs per journey). The allocation of the SD was changed for the different journeys in order to avoid an effect of location in the lorry. The front, middle and rear locations were as follows: SD3-SD2-SD1, for the first journey in winter; SD1-SD3-SD2 for the second journey in winter; SD2-SD1-SD3 for the first journey in summer; and finally SD2-SD3-SD1 for the second journey in summer.

The day before transport, all lambs were weighed and randomly assigned to one of the three SD studied. They were loaded early in the morning (at 8:00 am).

The lambs were loaded and unloaded individually by the same two experienced stockpersons in all transports. The transport vehicle was a three-axel rigid chassis with three floors and vents for natural ventilation located along the full length of its body, down both sides and on each floor. The left side of the ground floor was used for the current experiment. Study animals travelled alone in the lorry. The same driver drove the lorry in the four experimental transports. The transport route in the four trips was: at the start, lorry was driven for 25-30 min by a county road with poor quality surface at variable low speeds from the farm until it achieved the highway road. In the highway road lorry was driven for around 1 h, approximately constant speed of 80 km h^{-1}. Then, the truck was driven by a smooth secondary road for about 1 h, with a variable speed always lower than 80 km h^{-1}. Thereafter, the lorry travelled to abattoir during approximately 30 min at variable low speeds by a rough road. The weather conditions in both summer trips were partly cloudy and rained sporadically, in winter trips were cloudy with wind gusts and did not rain during the both journeys.

The lorry was equipped on the day prior to the transport trials. Spacers were placed on the truck to adjust the space allowance to the densities needed for the study, and two Hygrochron® sensors (Maxim Integrated Products, Inc., Silicon Valley, CA, USA) were installed to record the temperature and relative humidity, one was placed inside in the central area of the lorry at level of the animals, and the other one was installed outside of the lorry. Temperature-humidity index (THI) was calculated from the temperature and humidity data obtained from the inside sensor according to the formula suggest by West (1994) and used in lamb transport by Miranda de la Lama *et al.* (2012a).

Carcass and meat quality analyses

Immediately after transport, the lambs were unloaded, weighed and held in lairage for less than 1 h with access to fresh clean water. They were then held individually in a restrainer, electrically stunned and commercially slaughtered by severance of the carotid arteries and jugular veins. Hot carcass and liver weights (liver is usually included in suckling lamb carcasses) were recorded after slaughter and dressing. Initial pH was immediately measured in *M. longissimus*, between the 13th thoracic and 1st lumbar vertebrae, *M. semitendinosus* and *M. psoas major* using a penetrating electrode with temperature probe attached to a portable pH-meter (Crison® 507, Alella, Spain). The pH was also recorded at 45 min, 3 h and 24 h after slaughter. The carcasses were stored at 4°C for 24 h. After that, cold carcass weights were recorded in order to calculate dressing percentage (cold carcass weight/ LW·100) and chilling losses ((hot carcass weight-cold carcass weight)/hot carcass weight·100).

The *M. longissimus* was excised from both sides of the carcasses 24 h *post mortem*. The muscles from the left side were used for initial meat quality analyses (24 h *post mortem*). The *M. longissimus* from the right side were then placed on Styrofoam trays, over-wrapped using oxygen-permeable PVC film (thickness: 8 μm; oxygen transmission rate: 3,000 cc m^{-2} day^{-1} at 5°C) and stored in the dark for 5 days at 4°C (5 days of refrigerated storage).

All meat quality parameters were measured in both non-stored (initial) and after 5 days of refrigerated storage. The CIE $L^*a^*b^*$ colour was measured at the cut surface using a CM-2600d spectrophotometer (Minolta Co., Osaka, Japan; illuminant: D_{65}; visual angle: 10°; measurement aperture: 8 mm). The initial colour (at day 0) was measured after blooming for 1 h. Colour coordinates were expressed as L* (lightness), a* (redness), and b* (yellowness). Chroma (C*) and hue angle (h*) values were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and $h^* = \tan^{-1}(b^*/a^*)$, respectively. The relative proportion of meat pigments (metmyoglobin, deoxymyoglobin and oxymyoglobin) was calculated using the equations proposed by Krzywicki (1979).

The water holding capacity (WHC) was measured as pressed juice percentage, using the method described by Grau & Hamm (1953), and as cooking loss percentage. Cooking loss percentage was determined as the loss with respect to the initial weight after cooking the samples for 30 min in plastic bags immersed in a water bath at 75°C according to De la Fuente *et al.* (2010).

Texture analyses were performed on the cooked meat samples described above using a TA-XT2 Texture Analyser® (Stable Micro Systems, Surrey, UK) equipped with a Warner-Brazler blade (25 kg load cell and 2 mm s^{-1} crosshead speed). Samples with a 1 × 1 cm cross-section were cut parallel to the muscle fibre direction. The parameters measured were maximum shear force (kg cm^{-2}) and shear firmness (kg s^{-1} cm^{-2}).

Lipid oxidation was determined using the 2-thiobarbituric acid method described by Maraschiello *et al.* (1999) with thiobarbituric acid-reactive substance (TBARS). The results (average of two determinations for each sample) were expressed as mg malonaldehyde (MDA) kg^{-1} muscle.

Statistical analyses

Data were analyzed statistically using the GLM procedure of SAS package (SAS® 9.2, SAS Inst. Inc. Cary, NC, USA). A two way model with fixed effect of season (winter and summer) and SD (with three levels; SD1, SD2 and SD3) and their interaction effect (Season*SD) was applied. Replicate of trials was tested before applying the final statistical model and it was not significant ($p > 0.05$). The LW previous transport was used as a covariate and was removed from the model if it was not significant ($p > 0.05$). In addition, the effect of storage time (24 h *post mortem* and after 5 days of refrigerated storage) was tested with MIXED procedure, including in the model season and SD as fixed factors and storage time as repeated measure and the interactions amongst these three factors. The effect of refrigerated storage was explained when its interaction with season or SD were significant. Differences between means were determined using the Student-Newman-Keuls test ($p < 0.05$).

Results

In winter, temperatures inside the lorry ranged between 7.4 and 11.4°C during the first journey, with an average of 9.1 ± 0.1°C. Relative humidity values ranged between 60.1 and 79.9%, with an average value of 70.2 ± 0.5%. In the second journey, the average of temperature and humidity were 8.5 ± 0.2°C and 53.7 ± 0.8% respectively, with ranges of 3.5 to 14.5°C for temperature and 38.1 to 74.7% for humidity. In summer, the temperatures recorded inside the lorry during the first journey ranged between 11.5 and 16.0°C, with relative humidity percentages between 71.5 and 98.8% and averages of 12.8 ± 0.1°C and 82.5 ± 0.5% respectively. In the second journey, the average of temperature was 16.3 ± 0.2°C (maximum: 21.6°C and minimum: 13.5°C) and of humidity was 76.8 ± 0.4% (maximum: 83.5%, minimum: 62.6%). In winter, the mean THI in the first journey was 49.9 ± 0.09, with a minimum of 47.6 and a maximum of 53.6 and, in the second journey, the average of THI was 49.7 ± 0.34, with a range between 41.3 and 58.1. In the journeys carried out in summer, the average of THI were 55.2 ± 0.18 and 60.9 ± 0.24 in the first and second trip respectively, ranged between 53.1 to 60.8 in the first one and 56.5 to 68.2 in the second one.

Carcass quality

The effects of season and SD on the carcass quality parameters are shown in Table 1. No significant interactions between season and SD were found for any of the parameters studied. The LW parameters were affected by season, being higher in lambs transported in winter ($p < 0.05$) than those transported in summer ($p < 0.001$). The SD had no significant effect on either carcass quality parameters or LW ($p > 0.05$).

pH evolution

Table 2 shows the pH values for *M. longissimus*, *M. semitendinosus* and *M. psoas major* according to season and SD. Season affected initial pH (0 min) values ($p < 0.001$) in the three muscles studied; lambs transported in summer showing higher values than

Table 1. Carcass quality of suckling lambs in relation to season and stocking density

	Season		**Stocking density (SD)**[1]			**SE**[2]	***p*-value**[3]		
	Winter	**Summer**	**SD1**	**SD2**	**SD3**		**Season**	**SD**	**Season*SD**
Slaughter live weight[4] (kg)	12.77	12.79	12.83	12.78	12.73	0.43	NS	NS	NS
Hot carcass weight[4] (kg)	7.23	7.10	7.13	7.19	7.18	0.28	NS	NS	NS
Cold carcass weight[4] (kg)	7.05	6.99	6.98	7.04	7.05	0.26	NS	NS	NS
Dressing percentage (%)	55.17	54.65	54.39	55.03	55.31	1.66	NS	NS	NS
Chilling losses (%)	2.52	2.45	2.44	2.45	2.18	1.16	NS	NS	NS
Liver weight[4] (g)	355.6	315.5	335.5	326.2	344.8	46.3	***	NS	NS

[1] SD1: 0.08 m^2 animal^{-1}; SD2: 0.12 m^2 animal^{-1}; SD3: 0.20 m^2 animal^{-1}. [2] Standard error of the mean (n = 12). [3] NS: not significant; *** $p < 0.001$. [4] Live weight before transport was used as a covariate in those parameters.

Table 2. Values of pH in *M. longissimus, semitendinosus* and *psoas major* in relation to season and stocking density

	Season		Stocking density (SD)[1]			SE[2]	*p*-value[3]		
	Winter	Summer	SD1	SD2	SD3		Season	SD	Season*SD
M. longissimus									
0 min[4]	6.65	6.92	6.75	6.81	6.76	0.17	***	NS	NS
45 min	6.46	6.48	6.48	6.48	6.44	0.18	NS	NS	NS
3 h	6.15	6.18	6.09^y	6.20^x	6.21^x	0.17	NS	*	NS
24 h	5.67	5.73	5.69	5.71	5.71	0.20	NS	NS	NS
M. semitendinosus									
0 min[4]	6.30	6.54	6.32	6.49	6.46	0.25	***	NS	NS
45 min	6.19	6.24	6.12^y	6.27^x	6.26^x	0.20	NS	*	NS
3 h	6.01	6.03	5.98	6.07	6.01	0.17	NS	NS	NS
24 h[4]	5.74	5.65	5.67	5.73	5.69	0.15	**	NS	NS
M. psoas major									
0 min[4]	5.99	6.23	6.12	6.09	6.13	0.22	***	NS	NS
45 min	5.94	5.93	5.93	5.94	5.94	0.19	NS	NS	NS
3 h	5.84	5.81	5.82	5.82	5.84	0.19	NS	NS	NS
24 h	5.77	5.83	5.75	5.83	5.82	0.20	NS	NS	NS

[1] SD1: 0.08 m^2 animal^{-1}; SD2: 0.12 m^2 animal^{-1}; SD3: 0.20 m^2 animal^{-1}. [2] Standard error of the mean (n = 12). [3] NS: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. [4] Live weight before transport was used as a covariate in those parameters. ^{x,y} different letters within the same row indicate significant differences ($p < 0.05$) among stocking densities.

those transported in winter. No effect of season ($p > 0.05$) was found for pH measured at 45 min and 3 h. Only in *M. semitendinosus*, the final pH (pH_{24}) was affected by season ($p < 0.01$), being lower in lambs transported in summer than those transported in winter.

Concerning SD, the pH at 3 h (pH_{3h}) after slaughter in *M. longissimus* and pH at 45 min (pH_{45min}) in *M. semitendinosus* were affected by it ($p < 0.05$), with lower values in SD1 than SD2 or SD3.

Colour and meat pigments

The meat colour parameters for *M. longissimus,* initially (24 h *post mortem*) and after 5 days of refrigerated storage, in relation to season and SD, are shown in Table 3. Season affected most of the colour parameters studied both initially and after 5 days of storage. Lambs transported in summer had higher L*, a*, b* and C* values initially, and higher L*, b*, and C* values after 5 days of storage than those transported in winter. Significant interactions between storage time and season were found for parameters a* ($p < 0.05$), b* ($p < 0.05$), C* ($p < 0.01$) and h*($p<0.05$). Thus, a* increased with storage time in lambs transported in winter (from 1.1 to 2.5), whereas it remained constant in summer (from 2.7 to 2.4). The values of b* increased with storage time in both seasons, but this increment was greater in winter (from 9.2 to 11.6) than in summer (from 11.1 to 12.8). Similar interaction was found for C*, with values of 9.5 at 24 h *post mortem* and 12.0 after 5 days of refrigerated storage in winter, and 11.7 at 24 h *post mortem* and 13.2 after 5 days of refrigerated storage in summer transport. With respect to h*, in winter, the value decrease with storage time from 83.8 to 78.3, whereas in summer an increment (from 76.8 to 79.4) was found. Colorimetric parameters were not affected by stoking density ($p > 0.05$).

Table 4 shows the relative proportion of meat pigments in *M. longissimus* in terms of season and SD for both times of storage. Lambs transported in summer had higher metmyoglobin and oxymyoglobin proportion and lower deoxymyoglobin proportion than those transported in winter at 24 h *post mortem*. Significant interactions between season and storage time on metmyoglobin ($p < 0.05$), deoxymyoglobin ($p < 0.001$) and oxymyoglobin ($p < 0.01$) proportions were found. Thus, metmyoglobin proportion increased with storage time in winter (from 6.0 to 10.3) whereas, in summer, this proportion was similar (12.8 *vs.* 12.9 at 24 h *post mortem* and after 5 days of refrigerated storage, respectively). Deoxymyoglobin proportion decreased with

Table 3. Colour parameters of *M. longissimus* in relation to season and stocking density

	Season		Stocking density (SD)[1]			SE[2]	*p*-value[3]		
	Winter	Summer	SD1	SD2	SD3		Season	SD	Season*SD
24 h post mortem									
L*	43.8	46.8	45.8	45.9	44.2	3.68	***	NS	NS
a*	1.05	2.68	1.73	1.41	2.46	2.26	**	NS	NS
b*	9.18	11.10	10.14	10.12	10.16	0.89	***	NS	NS
C*	9.45	11.65	10.51	10.46	10.68	1.05	***	NS	NS
h*	83.8	76.8	80.5	83.2	77.2	11.81	*	NS	NS
5 days of refrigerated storage									
L*	47.1	50.7	49.2	49.4	48.1	2.8	***	NS	NS
a*	2.50	2.43	2.31	2.13	2.97	2.12	NS	NS	NS
b*	11.56	12.81	12.40	12.00	12.16	0.89	***	NS	NS
C*	12.01	13.19	12.76	12.33	12.72	1.11	***	NS	NS
h*	78.3	79.4	79.4	80. 5	76.8	9.07	NS	NS	NS

[1] SD1: 0.08 m^2 animal^{-1}; SD2: 0.12 m^2 animal^{-1}; SD3: 0.20 m^2 animal^{-1}. [2] Standard error of the mean (n = 12). [3] NS: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

storage time in both winter and summer, but the difference was greater in winter (from 93.4 to 68.7) than in summer (from 72.5 to 68.6). Oxymyoglobin proportion increased with storage time in both winter and summer, being the difference greater in winter (from 0.6 to 21.0) than in summer (from 14.7 to 18.5). The SD did not show any effect on meat pigment proportions.

Water holding capacity, texture and lipid oxidation parameters

The pressed juice, cooking loss, shear force, shear firmness and TBARS values are shown in Table 5. Season affected most of these parameters both initially and after 5 days of storage respectively. Pressed juice was higher ($p < 0.01$) in lambs transported in winter than those in summer initially, however, after 5 day of refrigerated storage, lambs transported in winter had lower pressed juice ($p < 0.001$) than lambs transported in summer. A significant interaction between season and storage time was found for pressed juice ($p < 0.001$). Thus, in winter pressed juice decreased with refrigerated storage (from 21.7 to13.7), whereas in summer the values were similar (19.8 *vs.* 18.4 at 24 h *post mortem* and after 5 days of refrigerated storage respectively). Pressed juice and cooking loss were not affected by SD ($p > 0.05$).

Table 4. Proportion (%) of meat pigments of *M. longissimus* in relation to season and stocking density

	Season		Stocking density (SD)[1]			SE[2]	*p*-value[3]		
	Winter	Summer	SD1	SD2	SD3		Season	SD	Season*SD
24 h post mortem									
Metmyoglobin	6.0	12.8	9.1	8.4	10.8	5.42	***	NS	NS
Deoxymyoglobin	93.4	72.5	81.9	85.9	80.9	13.91	***	NS	NS
Oxymyoglobin	0.6[b]	14.7	8.9	5.7	8.3	10.09	***	NS	NS
5 days of refrigerated storage									
Metmyoglobin	10.3	12.9	11.2	10.8	12.8	5.30	*	NS	NS
Deoxymyoglobin	68.7	68.6	66.6	70.5	68.9	19.12	NS	NS	NS
Oxymyoglobin	21.0	18.5	22.1	18.7	18.4	17.93	NS	NS	NS

[1] SD1: 0.08 m^2 animal^{-1}; SD2: 0.12 m^{-2} animal^{-1}; SD3: 0.20 m^2 animal^{-1}. [2] Standard error of the mean (n = 12). [3] NS: not significant; * $p < 0.05$; *** $p < 0.001$.

Table 5. Water holding capacity, texture parameters and lipid oxidation (TBARS) in M. longissimus in relation to season and stocking density

	Season		Stocking density (SD)[1]			SE[2]	*p*-value[3]		
	Winter	Summer	SD1	SD2	SD3		Season	SD	Season*SD
24 h post mortem									
Pressed juice (%)	21.7	19.8	20.5	21.0	20.7	2.82	**	NS	NS
Cooking loss (%)	30.6	29.2	29.6	30.7	29.4	4.37	NS	NS	NS
Shear force (kg cm^{-2})	7.95	5.34	6.80	6.81	6.34	1.77	***	NS	NS
Shear firmness (kg s^{-1} cm^{-2})	1.35	1.06	1.23	1.21	1.16	0.24	***	NS	NS
TBARS (mg MDA4 kg^{-1})	0.06	0.10	0.06	0.08	0.10	0.21	NS	NS	NS
5 days of refrigerated storage									
Pressed juice (%)	13.7	18.4	15.5	16.4	16.3	2.53	***	NS	NS
Cooking loss (%)	29.1	27.7	28.1	29.3	27.8	4.17	NS	NS	NS
Shear force (kg cm^{-2})	6.42	3.98	5.24	5.34	5.02	1.70	***	NS	NS
Shear firmness (kg s^{-1} cm^{-2})	1.13	0.86	1.00	1.01	0.97	0.19	***	NS	NS
TBARS (mg MDA[4] kg^{-1})	0.57	1.08	0.77	0.75	0.95	0.62	**	NS	NS

[1] SD1: 0.08 m^2 animal^{-1}; SD2: 0.12 m^2 animal^{-1}; SD3: 0.20 m^2 animal^{-1}. [2] Standard error of the mean (n = 12). [3] NS: not significant; ** $p < 0.01$; *** $p < 0.001$. [4] MDA: malonaldehyde.

Season of transport affected ($p < 0.001$) shear force and shear firmness, with meat from lambs transported in winter having higher values than those transported in summer initially (24 h *post mortem*) and after 5 days of storage. As expected, both parameters decreased as time of storage increased for both season of transport. The SD did not show any effect on these texture measures.

With regard to lipid oxidation, TBARS values were not affected by season of transport in meat at 24 h *post mortem*, but after 5 days of refrigerated storage, lambs transported in winter had lower TBARS values ($p < 0.01$) than those transported in summer. As expected, TBARS values increased with storage in both seasons, however no effect of SD was found.

Discussion

Carcass quality and liver weight

Pre-slaughter stress is associated with LW and carcass weight losses and depletion of muscle glycogen reserves (Warriss, 1993). In this sense, Kadim *et al.* (2010) observed on goats that animals transported at high ambient temperature (37°C average) had higher LW losses and lower carcass shrinkage than non-transported animal. However, in our study, carcass quality parameters have not been affected by season or SD, because the temperature occurred during transport in summer or winter were not extreme, with THI values, good indicator of stressful thermal climatic conditions, within the category considered as comfortable (< 70) (Silanikove, 2000). The lower liver weight found in animals transported in summer may indicate a greater mobilization of glycogen reserves from the liver. According to Jacob (2003), hepatic glycogen reserves are very susceptible to depletion due to nutritional deprivation, exercise or stress. In this sense, Miranda de la Lama *et al.* (2012a) indicated that longer days and high temperatures in summer favoured locomotor activity in lambs. Thus, this higher activity in summer might have affected to liver glycogen reserves due to its mobilization, reducing therefore liver weight. In fact, as discussed by Warriss (1990), the mobilisation of tissues to provide energy to maintain vital body functions could result in losses in the weight of liver. The SD did not affect carcass quality, in agreement with results found by De la Fuente *et al.* (2010) in suckling lambs.

pH evolution

One of the most important parameters used to evaluate meat quality is pH value that could be influenced by factors related to transport (Ruiz de la Torre *et al.*, 2001; Miranda de la Lama *et al.*, 2011) and by season (Kadim *et al.*, 2008; Miranda de la Lama *et al.*, 2011).

The initial pH values of *M. longissimus* were in the usually range, however, our results showed lower initial pH values in animals transported in winter than in those transported in summer for the three muscles studied. Similarly, Kadim *et al.* (2008) observed, in goat and sheep, that animals slaughtered in the hot season had higher initial pH than those slaughtered in the cool season (average 6.82 *vs.* 6.65). Our results indicated that the rate of glycogen metabolism was accelerated (Warris, 1990) in the lambs transported in winter. McVeigh *et al.* (1982) stated that glycogenolysis in skeletal muscle is activated by physiological stress. Notwithstanding, in our study, as commented above, THI for both seasons were lesser than 69, which can be considered as comfortable (Silanikove, 2000). Miranda de la Lama *et al.* (2012a) observed that lambs transported in winter experienced more stress response, having higher cortisol levels and ultimate pH than those transported in summer, with THI values within danger category (79-83) in both seasons. In our study, the ultimate pH in *M. semitendinosus* was affected by season with higher values in lambs transported in winter compared to those transported in summer; however ultimate pH in *M. psoas major* or *M. longissimus* were similar for both seasons. In contrast, Kadim *et al.* (2008) in lambs of 30-38 kg LW, found higher ultimate pH values in *psoas major* and *minor* muscles from animals transported during the warmer season due to a combination of high temperatures and stress prior to slaughter. Nevertheless, these authors, as in our work, noted that those differences in pH between seasons were reduced as the time *post mortem* progressed, being higher in initial pH than in final pH.

The SD did not have a great influence on the pH values in the muscles studied. Only, lower pH_{3h} in *M. longissimus* and pH_{45min} in *M. semitendinosus* were obtained for animals transported at a high SD compared to those transported at medium and low SD. In a previous study, carried out by De la Fuente *et al.* (2010) in suckling lambs, animals transported at high density (0.12 m^2 $lamb^{-1}$) had lower pH_{24h} compared to lambs transported at lower SD (0.20 and 0.25 m^2 $lamb^{-1}$). These differences could be because of transport of suckling lambs at high SD might produce a high energy demanding situation that consequently might affect the meat pH (Ruiz de la Torre *et al.*, 2001; Miranda de la Lama *et al.*, 2011). In this sense, De la Fuente *et al.* (2012) observed that the behaviour of suckling lambs during transport is affected by SD, with less animals lying at high SD than at low. Similarly in heavier lambs, Vargas (2009) reported a higher proportion of animals standing during transport at a high SD due to the limited space available to lie down. It is interesting to note that although we found some significant differences in pH values, they were within the normal range of pH reported by other authors for suckling lambs (Devine *et al.*, 2006; Santos *et al.*, 2007).

Colour and meat pigments

Meat colour, which is one of the most important criteria affecting consumers' initial choice, can be affected by factors related to pre-slaughter handling, such as transport (Warriss *et al.*, 1990). The values obtained in colorimetric parameters are consistent with pale meat, as befits suckling lambs fed exclusively on milk. Nevertheless lambs transported in winter had slightly darker meat colour, as indicates their low L* and C* values, compared to those transported in summer. Similar results have been reported in heavier lambs by Miranda de la Lama *et al.* (2012b) who found darker meat in lambs subjected to a direct transport in winter compared to those transported in summer. These authors attributed the darker colour in lambs transported in the cold season to high ultimate pH which could be consequence that this season was more stressful than the hot season. However, in our study, the pH_{24h} in *M. longissimus* was not significantly different between both seasons, although the pH_{0h} was lower in lambs transported in winter, this fact could have had consequences for the structure of the myofibrillar proteins and biochemical *post-mortem* process (Geay *et al.*, 2001), and therefore the meat colour might be affected. The effect of season on a*, b* and C* was less pronounced in measurements taken after 5 days of refrigerated storage than in those recorded at 24 h *post mortem*, which could be due to the fact that storage might attenuate any negative effects of transport on meat quality, as suggested previously by María *et al.* (2003).

It is well known that muscle colour depends on the pigment content and the chemical pigment form, which is affected by pH (Lindahl *et al.*, 2001; Mancini & Hunt, 2005). The proportions of metmyoglobin and oxymyoglobin are known to have a marked effect on the variation in L*, the proportion of metmyoglobin on the variation in a*, and the proportions of deoxymyoglobin and oxymyoglobin on the variation in b* (Lindahl *et al.*, 2001). In this sense, lower a* and b* values in lambs transported in winter were related to more deoxymyoglobin and lower oxymyoglobin proportion at the meat surface, *i.e.,* to a lower degree of myoglobin oxygenation (Lindahl *et al*., 2001). The proportion of deoxymyoglobin is related to both meat pH and the rate of early *post mortem* pH decrease, as a low pH affects the oxygen consumption rate within the meat as a result of either a decrease in activity or inactivation of enzymatic oxygen consumption systems (Rosenvold & Andersen, 2003). As was the case for the colorimetric parameters, the effect of season on meat pigment proportions varied according to the time the measurements were taken, with differences between seasons being more evident at 24 h *post mortem* than after 5 days of refrigerated storage.

No effect of SD on colour parameters was observed in either non-stored (initially) or stored meat, which is consistent with the little effect of SD on pH, one of the main parameters related to meat colour. The results of the current work are in the line of those obtained previously by De la Fuente *et al*. (2010) for suckling lambs transported for 30 min or 5 h at 0.12, 0.20 and 0.25 m^2 $animal^{-1}$.

Water holding capacity, texture and lipid oxidation parameters

The lower values of WHC, measured as pressed juice (higher proportion of juice expelled) in animals transported in winter than those transported in summer, that could be related to the lower initial pH in lambs transported in winter closer to the muscle protein isoelectric point (Touraille, 1994). This result is consistent with the findings of Kadim *et al*. (2008) in heavier sheep (30-38 kg carcass weight), who found higher expressed juice in animals slaughtered in cold than in hot season. An explanation of the lack of differences in cooking losses despite the differences in terms of pressed juice could be due to the more aggressive cooking treatment releases both bound and immobilized water, which may have minimized the effect of transport season.

As was the case in our study, in suckling lambs, De la Fuente *et al*. (2010) found no effect of density on any of the parameters used to estimate the water retention capacity, after either 24 h *post mortem* or after 5 days of cooling process.

Shear force and shear firmness were both affected by season, thus, lambs transported in winter had less tender meat than those transported in summer at 24 h *post mortem* and after 5 days of refrigerated storage. These results are in accordance with those obtained by Miranda de la Lama *et al*. (2012b) in heavier lambs, who found higher values for shear force and toughness parameters in meat from lambs transported in winter than for those transported in summer. These authors stated that the tougher meat from animals transported in winter was related to a higher biological cost of adaptation during this season. Similar results have been obtained in other species as beef cattle by Kadim *et al*. (2004) who suggest that lower ambient temperature could be related with higher glycogen content in the pre-rigor state and thus alter energy metabolism, which could be partially responsible of seasonal variation in the Warner-Bratzler parameters.

Living organisms have developed complex antioxidant defence systems that protect the body from oxidative damage (Halliwel & Gutteridge, 1996). Although there is normally a balance between antioxidant defence systems and free radicals, it has been reported that effort situations, such as exercise, and stress factors caused by transportation can decrease antioxidant capacity and result in increases in serum MDA concentrations (Onmaz *et al*., 2011). Thus, we found higher TBARS values in stored meat from animals transported in summer than in meat from those transported in winter could be due to the higher locomotive activity undertaken by the former. Furthermore, according to Evans (2000), increased lipid peroxidation may cause damage within the sarcoplasm cell membrane, thus leading to dysfunction of myofilament structures and impaired contraction which, in turn, could have affected WHC and texture values. It is possible that these differences are only observed after 5 days of refrigerated storage as the natural antioxidative system of the animal decreases with time after slaughter.

Considering these results, it can be concluded that liver weight and some of the meat quality parameters of suckling lambs were affected by season, with darker and harder meat obtained in those animals transported in winter than those transported in summer, but the values were within a normal range. However, the effect of season on colour and lipid oxidation was dependent on meat storage. Carcass and meat quality were essentially unaffected by the SD during transport. Therefore, under our experimental conditions the three SD studied appear to be suitable for suckling lambs transported for 3 h, the handling of suckling lambs before, during and after transport should not neglect mainly in those transports carried out in winter where its meat quality may be reduced.

Acknowledgments

This research was supported by an INIA project (RTA2005-00069-C2). We would like to thank the staff of both "Estación Tecnológica de la Carne" and "Magnus S.A." and its processing plant staff for their help and cooperation in this study. We would also like to thank the "Instituto Tecnológico Agrario" for the award of a PhD grant to M. Sánchez.

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