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Effect of space allowance during transport and fasting or non-fasting during lairage on welfare indicators in Merino lambs

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Abstract

A total of 72 male lambs of Merina breed were sampled in a 3×2 factorial design, testing three different space allowances treatment (SA) during transport [0.16 m²/animal (SAL; n=24); 0.20 m²/animal (SAM; n=24) and 0.30 m²/animal (SAH; n=24)] and two lairage treatments (TL) during 18 h previous slaughter [fasting (FAST; n=36) vs feeding (FEED; n=36)] on welfare physiological indicators. After transport, glucose and lactate dehydrogenase (LDH) were highest in SAM group and lowest in SAH one ($p<0.05$). SAL showed intermediate values for both parameters. SA did not affect the rest of the blood parameters studied. TL-FAST treatment decreased glucose values ($p<0.001$) while increased LDH ($p<0.001$). Fasting caused an increase ($p<0.05$) of Red Blood Cell Count values in SAM group. Feed deprivation did not affect cortisol or adrenaline values. Noradrenaline value was higher ($p<0.001$) in TL-FAST groups than in TL-FEED. In conclusion, under the conditions of this study, a range of space allowance during transport between 0.16 and 0.30 m²/lamb could be recommended without showing major changes on welfare physiological indicators; and feeding could be more appropriate than fasting during lairage.

Additional key words: sheep; blood; density; transportation; management pre-slaughter; feed-deprivation.

Abbreviations used: CK (creatine kinase); EDTA (ethylene diaminetetraacetic acid); FAST (fasting); FEED (feeding); FEED-SAH (feeding space allowance high); GLM (General Lineal Model); LDH (lactate dehydrogenase); RBC (Red Blood Cell Count); SA (space allowance treatment during transport); SAH (space allowance high); SAL (space allowance low); SAM (space allowance medium); TL (lairage treatment).

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Introduction

One of the main Spanish breeds of sheep, the Merina breed, is raised in the region of Extremadura (SW Spain) and it is the foremost commercial sheep breed in the world (<http://www.merino2014.com/conference.html>). Among the products obtained from this breed, lamb is a very valuable product, due to its highly appreciated organoleptic qualities (Tejeda *et al.*, 2008). The lambs are weaned at 45 days and then fed with a commercial concentrate and cereal straw *ad libitum* until slaughter (20–30 kg live weight).

Many factors relate directly to pre-slaughter stress, such as transport, fasting, conditions in the abattoir

which may affect meat quality (Sañudo *et al.*, 1998). Animal transport is an important phase in all systems of meat production (Ljungberg *et al.*, 2007). Transportation by road is the most common method used for lambs and causes changes in many blood parameters as a consequence of stress response (Knowles, 1998). According to Broom (2008), space allowed during transport is one of the most important factors influencing animal welfare. Protection of animals during transport is a legal requirement according to European law (EC, 2004) to prevent injury or undue suffering and to ensure appropriate conditions to meet their biological needs. A considerable amount of research has been conducted on the stressfulness of transportation on

several sheep breeds [Tadich *et al.* (2009) in the Corriedale breed; Miranda-de la Lama *et al.* (2010a, 2011, 2012) in the Rasa Aragonesa breed; De la Fuente *et al.* (2010, 2012) in the Assaf breed].

Lairage of animals at the slaughterhouse is a common commercial practice that helps livestock recover from transportation stress prior to slaughter (Kannan *et al.*, 2000). Fasting during this period is important for reducing gut contents prior slaughter resulting in a reduced risk of carcass contamination (Gregory & Grandin, 1998). However, it is suggested that provision of feed during lairage could reduce the stress in the animals (Gonyou, 2012). Some studies have considered the effect of lairage on lamb welfare, including time of lairage (Díaz *et al.*, 2014), with or without lairage (Liste *et al.*, 2011).

A variety of welfare indicators can be used to assess the welfare of animals (Broom, 2000). Many physiological parameters have been proposed to evaluate the effects of stress previous slaughter, such as hematological parameters (Liotta *et al.*, 2007); hormones (Mellor *et al.*, 2002), glucose (Tadich *et al.*, 2009) or tissular damage quantified by the change in creatinine kinase and lactate dehydrogenase (Ekiz *et al.*, 2012).

Stressors immediately prior to slaughter, including transport and lairage conditions can have a cumulative effect. To date, no studies have examined the effects of space allowance during transport and fasting or feeding during the lairage prior to slaughter on lamb welfare in this breed. This information would help to recommend appropriate management during the stay of lambs at the abattoir before slaughter. Therefore, the aims of this study were to investigate: (1) the effect of space allowance (SA: low, medium or high) during transport; (2) the management during lairage (TL: fasting vs feeding) on the haematological, hormonal and biochemical blood parameter values for Merina breed lambs; (3) to determine the best combination (space allowance during transport and management during lairage at the slaughterhouse) to obtain the highest animal welfare before slaughter.

Material and methods

Study description

Seventy two Merina breed male lambs (28.03 ± 0.05 kg, 90 days old) were used in this trial. Lambs were weaned at 45 days after birth and then fed a commercial concentrate (17% crude protein), containing cereals, soya, calcium carbonate, sodium chloride, mineral-vitamin mix and cereal straw *ad libitum*. All

handling practices were carried out according to statements of the Directive 2010/63/EU (EC, 2010) with regard to the protection of animals used in research and for scientific purposes.

The study was carried out in spring, in two journeys that were performed in April and May, respectively (both in sunny conditions and without rain). Values of the temperature and relative humidity were registered using a WatchDog 15–Temperature/Humidity Monitor. Animals ($n=36$ per journey) were loaded (starting at 8 am, 7°C average temperature during loading) randomly in the middle floor and in both sides of the lorry, according to the three spaces allowances: 0.16 m²/animal (SAL; $n=24$; 12/journey); 0.20 m²/animal (SAM; $n=24$; 12/journey) and 0.30 m²/animal (SAH; $n=24$; 12/journey). The same vehicle was used in both journeys.

Lambs were transported by paved roads (334 km, ~ 5½ h) from the farm (Cabeza del Buey, Badajoz, Spain; 38° 43' N) to the abattoir (Tarancón, Cuenca, Spain; 40° 0' N) along with other lambs not included in this study. The mean temperature and relative humidity recorded during transport were similar for both journeys, 11°C and 56% respectively (min. temp. 10.2°C and 13°C at the beginning and max. temp. 14°C and 16°C at the end of the journey for the first and second journey, respectively). The lorry had two axles and three floors, natural ventilation along the full length of the truck body sides and on each floor, a hydraulic elevator for loading and unloading. When animals arrived to the slaughterhouse were unloaded and rested in lairage for 18 h previous slaughter.

Animals in the slaughterhouse and in each group of density during transport were split randomly in two groups: one group of animals remained in fasting (FAST; $n=36$) during lairage, while the other group (FEED; $n=36$) were fed *ad libitum* the same commercial concentrate as on the farm). During lairage all animals remained in a waiting area, covered with a roof, at a density of 0.36 m² per animal and receiving water *ad libitum*. The average temperature at lairage was 14.7°C with a relative humidity of 62% (first journey: max. temp.=18.3°C, min. temp.=7.3°C; second journey: max. temp.=20.9°C, min. temp.=12.7°C). After lairage, lambs were slaughtered using standard commercial procedures.

Blood sampling

Blood samples were taken by trained personnel by external jugular venipuncture according to Linares *et al.* (2008) at three different times. The first sample used to determine the basal blood parameter concentrations

was taken on the farm in resting conditions; a second sample was taken immediately after unloading the animals to evaluate the effect of space allowance during transport. The final sample was collected after 18 h lairage, to determine the effect of the lairage treatment (feeding or fasting) before slaughter. Approximately 20 seconds were necessary for each extraction, and in all samples a 0.8×25 mm bevelled needle (Terumo Neolus, Belgium), 5 mL syringe (BD Discardit™, Spain) were used; 5 mL of blood was extracted to fill 1 mL tubes containing EDTA (ethylene diaminetetraacetic acid) for haematology and catecholamine determination and 4 mL tubes without additive for cortisol and biochemical parameter concentrations measurements. Blood samples were maintained at 2°C in a portable refrigerator until they arrived at the clinical analysis laboratory. The tubes were centrifuged at 4000 rpm for 5 min and frozen and kept at -18°C until processed.

The following physiological indicators were analysed:

- *Haematological*: Red Blood Cell (RBC), haemoglobin, haematocrit and leucocytes, were measured with an electronic haematological analyser (ABX Micros 60, Horiba ABX, France).
- *Hormonal*: Cortisol, adrenaline and noradrenaline. The determination of total cortisol concentration was carried out through a competitive enzyme assay (EIA, RADIM, Pomezia, Italy). The assay sensitivity was 5 ng/mL. The inter- and intra-assay coefficients of variation were 6% and 5%, respectively. The catecholamines were analysed using a competitive enzyme immunoassay kit (Cat Combi-Adrenaline-Noradrenaline ELISA, EIA-4309, DRG Instruments GmbH, Germany). The sensitivity in the analyses was 11 pg/mL for adrenaline and 44 pg/mL for noradrenaline. The inter- and intra-assay coefficients of variation were 14% and 11% for adrenaline and 12% and 13% for noradrenaline.
- *Biochemical*: Glucose, total protein, creatinine, lactate dehydrogenase (LDH), creatine kinase (CK) and lactate, were determined using a clinical system autoanalyzer (Synchron CX4 delta, Beckman Coulter Inc, USA).

Statistical analysis

Data were analysed with the Statistical Package SPSS 19.0 (IBM, 2010). First a Shapiro-Wilk test was carried out to check the normality and homogeneity of variance of all parameters. Then the repeated measured

ANOVA was carried out to determine the effects of space allowance treatment during transport on haematological, hormonal and biochemical blood parameters and the differences with the basal values on farm. A Tukey's test at a significance level of $p < 0.05$ was carried out to check the differences between pairs of groups.

A General Linear Model (GLM) was used to examine the effects of space allowance treatment during transport (low, medium, high) and treatment during lairage [Fasting (TL-FAST) or Non Fasting (TL-FEED)] and their interactions on haematological, hormonal and biochemical blood parameters after lairage. In addition a Tukey's test at a significance level of $p < 0.05$ was carried out to check the differences between pairs of groups [space allowance treatment (SA) – treatment during lairage (TL)].

A stepwise discriminant function analysis was carried out to select a linear combination of the independent parameters that best allowed differentiating among the SA groups during transport. A second discriminant analysis was carried out to differentiate among treatment groups during lairage. This last analysis was defined by two canonical discriminant functions which were illustrated by means of a dispersion diagram.

Results

Effect of space allowance during transport (SA)

Physiological parameters values for on farm and after transport per SA are showed in Table 1. Journey did not affect any blood parameter values on farm or after transport in each SA. In the present work haematological parameters after transport did not differ significantly from values detected at the farm. SA during transport did not affect these parameters.

After transport, the SAM group showed the highest cortisol value (142.10 nmol/L) while the lowest was found at the SAH (58.48 nmol/L), while SAL group showed intermediate values (101.53 nmol/L) for this parameter. However, there were not significant differences among SA treatments and values were similar to the values found on farm.

In general, adrenaline concentration was lower on farm than after transport but only significant differences ($p < 0.05$) were found between SAL groups (469.51 nmol/L on farm vs 662.59 nmol/L after transport). SA treatment during transport did not affect adrenaline concentration. Noradrenaline values on farm were similar to the values after transport ($p > 0.05$).

Table 1. Blood parameters in Merina breed lambs on farm and after transport (means \pm SE)

Blood parameters	On farm (basal blood values)			After transport (SA)			ANOVA
	SAL (0.16 m ² /lamb)	SAM (0.20 m ² /lamb)	SAH (0.30 m ² /lamb)	SAL (0.16 m ² /lamb)	SAM (0.20 m ² /lamb)	SAH (0.30 m ² /lamb)	
<i>Haematological parameters</i>							
RBC (10 ⁶ /mm ³)	9.82 \pm 0.21	9.55 \pm 0.41	9.65 \pm 0.23	10.37 \pm 0.20	10.23 \pm 0.25	10.43 \pm 0.28	NS
Haemoglobin (g/L)	0.70 \pm 0.01	0.72 \pm 0.01	0.70 \pm 0.01	0.74 \pm 0.01	0.73 \pm 0.01	0.74 \pm 0.01	NS
Hematocrit (%)	32.05 \pm 0.90	30.98 \pm 1.44	31.24 \pm 0.90	33.69 \pm 0.88	33.31 \pm 0.86	34.23 \pm 1.11	NS
Leucocytes (10 ³ /mm ³)	96.23 \pm 3.33	98.80 \pm 2.82	97.46 \pm 3.51	100.78 \pm 2.51	98.69 \pm 4.13	103.34 \pm 3.81	NS
<i>Hormonal parameters</i>							
Cortisol (nmol/L)	99.51 \pm 12.41	81.51 \pm 12.34	56.55 \pm 9.89	101.53 \pm 24.88	142.10 \pm 47.08	58.48 \pm 13.84	NS
Adrenaline (nmol/L)	469.51 \pm 37.69 ^a	517.67 \pm 46.84 ^{ab}	550.28 \pm 29.19 ^{ab}	662.59 \pm 45.56 ^b	627.57 \pm 53.97 ^{ab}	636.50 \pm 52.03 ^{ab}	*
Noradrenaline (nmol/L)	2562.81 \pm 228.83	2404.74 \pm 233.17	2469.40 \pm 257.71	2011.82 \pm 217.77	2387.50 \pm 332.03	2837.03 \pm 272.94	NS
<i>Biochemical parameters</i>							
Glucose (mmol/L)	4.43 \pm 0.18 ^a	4.37 \pm 0.21 ^a	4.77 \pm 0.19 ^{ab}	5.34 \pm 0.17 ^{bc,mn}	5.60 \pm 0.18 ^{c,n}	4.99 \pm 0.17 ^{ab,m}	***
Total protein (g/L)	52.32 \pm 2.92 ^a	55.07 \pm 2.48 ^a	62.33 \pm 2.83 ^{ab}	62.46 \pm 2.55 ^{ab}	68.07 \pm 3.67 ^b	67.87 \pm 3.46 ^b	***
Creatinine (μ mol/L)	60.50 \pm 3.94	67.34 \pm 4.85	66.76 \pm 3.32	66.34 \pm 3.42	71.49 \pm 4.70	68.95 \pm 4.10	NS
LDH (U/L)	274.65 \pm 14.66 ^a	314.00 \pm 23.63 ^{ab}	306.26 \pm 26.43 ^{ab}	350.91 \pm 21.26 ^{ab,mn}	398.96 \pm 32.61 ^{b,n}	296.52 \pm 20.38 ^{a,m}	**
CK (U/L)	129.48 \pm 20.84	130.78 \pm 24.47	122.95 \pm 27.45	239.05 \pm 63.38	206.10 \pm 32.16	244.25 \pm 56.39	NS
Lactate (g/L)	0.23 \pm 0.02	0.25 \pm 0.02	0.20 \pm 0.02	0.22 \pm 0.02	0.25 \pm 0.03	0.19 \pm 0.01	NS

SAL, SAM and SAH: low, medium and high space allowance during transport, respectively. SE: standard error. ^{a,b}: Values in the same row with different superscripts are significantly different ($p < 0.05$). ^{m,n}: Values in the same row with different superscripts are significantly different ($p < 0.05$) due to space allowance during transport. ANOVA: Significant differences among means for the same blood parameter. *, **, ***: indicate significance levels at 0.05, 0.01 and 0.001, respectively. NS: not significant; LDH: lactate dehydrogenase; CK: creatine kinase.

ANOVA test showed significant differences on glucose ($p < 0.001$), total proteins ($p < 0.001$) and LDH activity ($p < 0.01$). In general the lowest values for these serum indicators were found on farm and the highest in SAM group after transport. Tukey test showed significant differences ($p < 0.05$) among SA groups in glucose concentration and in the LDH activity: SAH one had the lowest values (4.99 mmol/L; 296.52 U/L respectively) with significant differences with SAM (5.60 mmol/L, 398.96 U/L respectively). The SAL group (0.16 m²/lamb) showed intermediate values for these biochemical parameters (5.34 mmol/L, 350.91 U/L respectively). However, total proteins, creatinine, CK and lactate values were not affected by the space allowance during transport.

The discriminant analysis showed that only the LDH activity marked the difference among SA groups. The centroid values were -0.124, 0.430 and -0.378 for SAL, SAM and SAH respectively; eigen value: 0.125; canonical correlation: 0.334.

Haematological, hormonal and biochemical parameters after lairage

Table 2 shows the values of blood parameters in each group of animals after lairage. Respect to haemato-

logical parameters, a significant effect ($p < 0.05$) of treatment during lairage was found on RBC, with significant differences ($p < 0.05$) between SAM groups. Cortisol after 18 h ranged between >65 nmol/L (TL-FAST-SAH) and <111 nmol/L (TL-FEED-SAM). The highest value of adrenaline (763.45 nmol/L) was found in TL-FAST-SAM group. There was a strong effect ($p < 0.001$) of lairage treatment on noradrenaline, with highest values in groups TL-FAST-SAL (2816.46 nmol/L) and TL-FAST-SAM (3171.40 nmol/L).

A significant effect of treatment during lairage was found on glucose ($p < 0.001$), total proteins ($p < 0.01$), creatinine ($p < 0.01$) and LDH activity ($p < 0.001$). In general, fasting during lairage decreased glucose values, while increased total proteins (in SAM group), creatinine and LDH activity (in SAH groups).

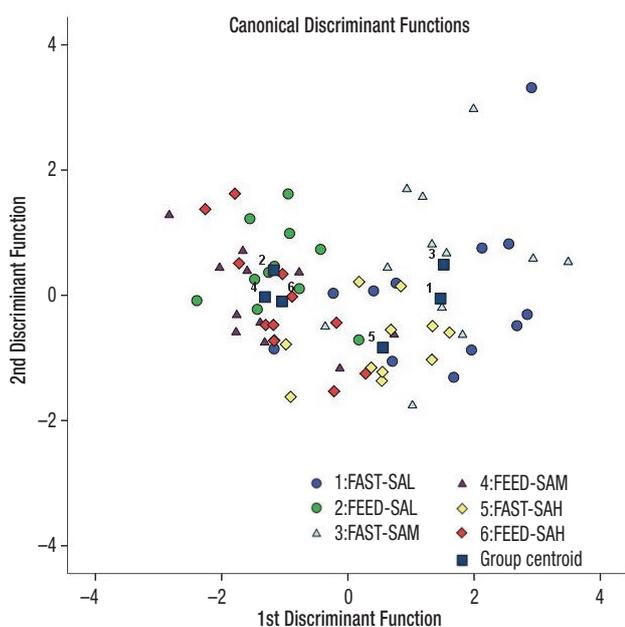
The discriminant analysis (Table 3) obtained two discriminant functions. The first explained the maximum of the existing differences among fasting groups (1: FAST-SAL, 3: FAST-SAM and 5: FAST-SAH) and no fasting groups (2: FEED-SAL, 4: FEED-SAM and 6: FEED-SAH), while the second function (only 10.3% of variance) discriminated between groups 2 and 3 from all other groups (1, 5, 4, 6). This analysis found that the parameters that truly marked the difference among groups were noradrenaline and glucose (the standard-

Table 2. Blood parameters in Merina breed lambs after lairage treatment (TL), fasting or feeding (means \pm SE).

Blood parameters	Fasting (TL-FAST)			Feeding (TL-FEED)			GLM		
	SAL	SAM	SAH	SAL	SAM	SAH	SA	TL	SA \times TL
<i>Haematological parameters</i>									
RBC ($10^6/\text{mm}^3$)	10.39 \pm 0.17 ^{xy}	11.02 \pm 0.25 ^y	10.59 \pm 0.39 ^{xy}	10.40 \pm 0.24 ^{xy}	9.73 \pm 0.33 ^x	10.19 \pm 0.36 ^{xy}	NS	*	NS
Haemoglobin (g/L)	0.73 \pm 0.01	0.76 \pm 0.02	0.73 \pm 0.02	0.74 \pm 0.01	0.72 \pm 0.02	0.74 \pm 0.02	NS	NS	NS
Hematocrit (%)	33.60 \pm 0.70	36.34 \pm 0.85	35.46 \pm 1.42	34.68 \pm 1.04	31.59 \pm 1.29	33.05 \pm 1.46	NS	NS	NS
Leucocytes ($10^3/\text{mm}^3$)	109.31 \pm 1.77	108.63 \pm 2.24	109.72 \pm 2.65	105.45 \pm 3.14	100.57 \pm 4.41	102.09 \pm 4.17	NS	NS	NS
<i>Hormonal parameters</i>									
Cortisol (nmol/L)	86.93 \pm 10.46 ^{xy}	93.71 \pm 10.64 ^{xy}	64.91 \pm 6.41 ^x	75.27 \pm 9.07 ^{xy}	110.68 \pm 12.91 ^y	102.48 \pm 15.10 ^{xy}	NS	NS	NS
Adrenaline (nmol/L)	575.83 \pm 84.63 ^{xy}	763.45 \pm 88.32 ^y	472.75 \pm 36.10 ^x	469.84 \pm 39.69 ^x	605.61 \pm 46.11 ^{xy}	621.93 \pm 46.23 ^{xy}	*	NS	NS
Noradrenaline (nmol/L)	2816.46 \pm 412.73 ^y	3171.40 \pm 333.53 ^y	1573.01 \pm 235.19 ^x	1291.38 \pm 179.19 ^x	831.39 \pm 155.65 ^x	1006.17 \pm 106.14 ^x	*	***	*
<i>Biochemical parameters</i>									
Glucose (mmol/L)	3.62 \pm 0.18 ^x	3.79 \pm 0.18 ^x	3.77 \pm 0.13 ^x	4.96 \pm 0.13 ^y	4.84 \pm 0.21 ^y	4.70 \pm 0.22 ^y	NS	***	NS
Total protein (g/L)	56.89 \pm 4.26 ^x	72.33 \pm 4.68 ^y	67.08 \pm 3.07 ^{xy}	56.73 \pm 1.75 ^x	55.96 \pm 1.47 ^x	58.22 \pm 2.01 ^x	NS	**	NS
Creatinine ($\mu\text{mol/L}$)	65.78 \pm 2.07 ^x	80.81 \pm 5.7 ^{xy}	105.20 \pm 11.70 ^y	80.69 \pm 1.91 ^{xy}	80.77 \pm 3.63 ^{xy}	77.55 \pm 2.88 ^x	NS	**	*
LDH (U/L)	370.50 \pm 17.09 ^{xy}	454.67 \pm 34.32 ^y	435.33 \pm 39.04 ^y	272.18 \pm 16.48 ^x	321.82 \pm 43.95 ^{xy}	252.09 \pm 33.07 ^x	NS	***	NS
CK (U/L)	237.09 \pm 54.34	181.18 \pm 54.12	294.36 \pm 90.48	234.30 \pm 73.89	184.70 \pm 35.87	181.73 \pm 53.14	NS	NS	NS
Lactate (g/L)	0.23 \pm 0.06	0.27 \pm 0.03	0.26 \pm 0.06	0.17 \pm 0.02	0.19 \pm 0.02	0.17 \pm 0.02	NS	NS	NS

SAL, SAM and SAH: low, medium and high space allowance treatments during transport, respectively. SE: standard error. ^{x,y}: values in the same row with different superscripts are significantly different ($p < 0.05$). GLM: General Lineal Model. *, **, ***: indicate significance levels at 0.05, 0.01 and 0.001, respectively. NS: not significant; LDH: lactate dehydrogenase; CK: creatine kinase.

ized coefficient of the first canonical function were 0.670 for glucose and 0.680 for noradrenaline while on the second one these values were 0.748 and 0.739, respectively). A dispersion diagram (Fig. 1) explains,

**Figure 1.** Dispersion diagram of the different groups of Merino lambs during lairage in the canonical discriminant functions.**Table 3.** Discriminant analysis of the effect of the lamb-type group (space allowance during transport-treatment during lairage): Canonical discriminant functions.

	Functions	
	1	2
<i>Standardized coefficient</i>		
Glucose	-0.670	0.748
Noradrenaline	0.680	0.739
Eigen-value	1.686a	0.194a
% of Variance	89.7	10.3
% Cumulative	89.7	100.0
Canonical correlation	0.792	0.403
<i>Centroid values</i>		
1: FAST-SAL	1.468	-0.052
2: FEED-SAL	-1.175	0.400
3: FAST-SAM	1.518	0.489
4: FEED-SAM	-1.315	-0.028
5: FAST-SAH	0.555	-0.835
6: FEED-SAH	-1.043	-0.097

^a The two first canonical discriminant functions had been used in the analysis. SAL, SAM and SAH: Low, medium and high space allowance during transport, respectively. FAST: Fasting during lairage. FEED: Feeding during lairage.

in graphical form, the results obtained in the statistical procedure: Groups 1, 3 and 5 (FASTING) are located in the area that corresponds to the positive value of the first function (with noradrenaline and glucose as main parameters). In regard to the second function (with the same main parameters), G2 (FEED-SAL) and G3 (FAST-SAM) are located in the area that corresponds to the positive values of this function while the rest of the groups (G1, G4, G5 and G6) are located in the negative part.

Discussion

Many critical points previous slaughter (such as transport or lairage conditions) may compromise lambs welfare (Miranda-de la Lama *et al.*, 2010b). However, sheep reaction to transport is affected by factors such as breed (Hall *et al.*, 1998) or age/weight (Bórnez *et al.*, 2009). Research on space allowances has been published for sheep (Cockram *et al.*, 1996; De la Fuente *et al.*, 2010, 2012; Knowless *et al.*, 1998; on Suffolk \times Greyface, suckling Assaf lambs, and shorn and unshorn lambs, respectively). There is little information about the effects of space allowance during transport and the lairage conditions before slaughter on welfare for Merino lambs used in the present study.

Effect of space allowance on blood parameters

Similar values were found on haematological parameters among the different SA groups. Miranda-de la Lama *et al.* (2011) found a similar number of RBC, leucocytes and hematocrit in Aragonesa lambs at similar age (100 days) and weight (25 kg) transported for 3 h. Our results could indicate that SA during transport is not a factor that affects these blood parameters. According to Mellor *et al.* (2002) cortisol and adrenaline values increase with emotional anxiety, while noradrenaline seems to be related to physical stress. In addition, the cortisol response to handling and transport depends upon the species and breed studied (Hall *et al.*, 1998). In this study the space allowance had no significant effect on cortisol values, results that are in agreement with Cockram *et al.* (1996) and De la Fuente *et al.* (2012). Our results showed a significant increase in adrenaline concentration after transport in SAL group but not corresponding increase in noradrenaline, in agreement with Åkerstedt *et al.* (1983). These findings imply that a space allowance between 0.16 and 0.30 m²/lamb did not cause a significant hormonal variation in lambs transported under the conditions provided in the present study.

Our findings showed that plasma glucose activity was influenced by space allowance. According to the results of Bórnez *et al.* (2009) in Manchega lambs and Tadich *et al.* (2009) in Corriedale breed, the highest values found in glucose at medium-SA could be associated with a high stress level. However, De la Fuente *et al.* (2012) did not find any effect of space allowed in this indicator in Assaf breed lambs. A different reaction to SA could be due to both lamb ages and sheep breeds.

SA had not effect on total protein values, results that are consistent with the findings by Cockram *et al.* (1996). Increased creatinine levels have been associated to dehydration (Montané, 2002). Pollard *et al.* (2002) in red deer subdued to different treatments previous to slaughter, indicated that the creatinine concentration was not a useful parameter to measure animal welfare, which is in agreement with the results found in the present study.

On the other hand, no differences for lactate values were detected among SA treatments, although other authors such as Pollard *et al.* (2002) or Miranda-de la Lama *et al.* (2011) found an increase for this welfare indicator related to stress. According to Grandin (1997) genetic factors, such as temperament, interact in complex ways with an animal's previous handling experiences and learning determining future reactions during a particular handling procedure.

High levels of serum LDH or/and CK have been correlated with muscle tissue damage as well as with vigorous exercise (Knowless, 1998; Miranda-de la Lama *et al.*, 2010a). Broom & Fraser (2007) indicated that CK activity could be considered as an indicator that permits determining appropriate minimum acceptable space allowances for transported animals. Although, in our study, no significant effects of space allowance during transport were detected on CK activity, an increase in LDH activity after transport, with highest values for the SAM group, were detected. Ibáñez *et al.* (2002) found a higher LDH activity in lambs transported at low (4 lambs/m²) than at high stocking density (8 lambs/m²) which is in agreement with our results. On the other hand, Grandin (2000) indicated that sheep may not need to lie down during short journeys. This could explain the absence of differences among SAH and SAL groups on LDH activity.

SA is an economical important factor, because reducing space can reduce costs of transport. In general, smaller space allowances lead to lower unit costs of transport since more animals can be carried in the vehicle (SCAHAW, 2002). A range of 0.16 to 0.30 m²/lamb could be acceptable during transport but investigation on behaviour will be required.

Effect of handling during lairage on animal welfare

In agreement with the findings by De Boer *et al.* (1989), fasting induced a rise in catecholamine levels, particularly of noradrenaline. In addition, catecholamine produced a contraction of the spleen with the subsequent increase in blood red cells values (Marco & Lavín, 1999). For this reason, the highest RBC values in the FAST-SAM group after lairage could be associated to the higher catecholamine value found in this group. However, our results contrast with the lack of variations in haematological values reported by Liste *et al.* (2011). Perhaps the different values in noradrenaline sampled after lairage could be related to the significant interaction SA \times TL that was observed and could explain the results on RBC.

Increase in cortisol concentration has been associated with feed-deprivation stress in Merino lamb (Zimmerman *et al.*, 2013), which contrasts with the results of cortisol found in the present paper. However, cortisol levels can vary greatly as it is a time-dependent response with high individual variability and therefore, comparisons among studies must be done with caution.

Glucose metabolism decreases due to the decline of propionate production, the major precursor for gluconeogenesis, in the rumen caused by low feed intake (Bergman, 1975). The lowest plasma glucose values found after lairage at the FASTING groups could be useful as an indicator of the intensity of stress, which is in agreement with the results by Kannan *et al.* (2000) for goats. The higher levels of glucose found in the present study when animals had access to feed during lairage (similar values to the basal conditions, on farm) could indicate recuperation overnight.

Liste *et al.* (2011) indicated that CK increase could be associated with a feed deprivation during lairage. However, in our study CK levels were similar across all TL groups, as found by Zimmerman *et al.* (2011). Gupta *et al.* (1999) associated the LDH decrease with feed deprivation in equids, which contrast with our results. Obviously this could respond to the different physiology of these two species. In addition, lairage without feed represented an additional stress, especially in lambs of SAM group. For this reason it is speculated that some physiologic parameters such as LDH increased in this treatment (SAM-TLFAST). This could indicate cumulative effects of the transport and lairage conditions.

With regard to the results of the discriminant analysis, the most notable data showed that this analysis only included the glucose and noradrenaline as the main parameters that allow differentiating among lairage treatments. In addition, the highest noradrenaline con-

centration and lowest plasma glucose values found after lairage in the FASTING groups could be useful as indicators of the intensity of stress, as found for goats (Kannan *et al.*, 2000).

Lairage allows the animals to recover from the stress endured during transport and consequently to improve meat quality (Rabaste *et al.*, 2007). Feed deprivation during lairage is a common practice to reduce gut contents, therefore minimizing the risk of carcass contamination. However, the results of this study indicate that fasting cause some physiological changes which would be indicative of compromised welfare. In addition, after 18 h lairage with access to feed, some physiologic parameters associated with stress (such as glucose, noradrenaline) or physical fatigue (such as LDH) returned to basal (farm) levels. Therefore, these results suggest that feeding during lairage could be more appropriate than fasting, especially when animals are stressed after transport, since it seems to allow overnight recovery. Nonetheless, it is not clear whether these changes are indicative of adaptation to feed deprivation, a sign of metabolic depletion or a compromised welfare. Though recommended from these results, the decision of feeding or fasting would also depend on other factors such as the economic cost, carcass hygiene or meat quality.

Some recommendations to meat industry could be given on the basis of the information obtained in this paper: From welfare standpoint a range of space allowance during transport of between 0.16 and 0.30 m²/lamb could be recommended without showing major changes on welfare physiological indicators. Feeding during lairage could be more appropriate than fasting, especially when animals showed more stress after transport, since it allows recuperation overnight.

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References

- Åkerstedt T, Gillberg M, Hjemdahl P, Sigurdson K, Gustavsson I, Daleskog M, Pollare T, 1983. Comparison of urinary and plasma catecholamine responses to mental stress. *Acta Physiol Scand* 117(1): 19-26. <http://dx.doi.org/10.1111/j.1748-1716.1983.tb07174.x>

- Bergman EN, 1975. Production and utilization of metabolites by the alimentary tract as measured in portal and hepatic blood. In: *Digestion and metabolism in the ruminant*; McDonald IW, Warner ACI (eds.). pp: 292-305. The University of New England Publishing Unit, Armidale, Australia.
- Bórnez R, Linares MB, Vergara H, 2009. Haematological, hormonal and biochemical blood parameters in lamb: Effect of age and blood sampling time. *Livest Sci* 121(2): 200-206. <http://dx.doi.org/10.1016/j.livsci.2008.06.009>
- Broom DM, 2000. Welfare assessment and welfare problem areas during handling and transport. In: *Livestock handling and transport*; Grandin T (ed.), 2nd edn. pp: 43-62. CABI Publ., Wallingford, UK. <http://dx.doi.org/10.1079/9780851994093.0043>
- Broom DM, 2008. The welfare of livestock during road transport. In: *Long distance transport and welfare of farm animals*; Appleby MCV, Cussen L, Garcés LA, Lambert JT (eds.). pp: 157-181. CABI Head Office, Wallingford, UK. <http://dx.doi.org/10.1079/9781845934033.0157>
- Broom DM, Fraser AF, 2007. *Domestic animal behaviour and welfare*, 4th edn. CAB Intnal, Wallingford, UK. 540 pp. <http://dx.doi.org/10.1079/9781845932879.0000>
- Cockram MS, Kent JE, Goddard PJ, Waran NK, McGilp IM, Jackson RE, Muwanga GM, Prytherch S, 1996. Effect of space allowance during transport on the behavioural and physiological responses of lambs during and after transport. *Anim Sci* 62(3): 461-477. <http://dx.doi.org/10.1017/S1357729800015009>
- De Boer SF, Koopmans SJ, Slangen JL, Van Der Gugten J, 1989. Effects of fasting on plasma catecholamine, corticosterone and glucose concentrations under basal and stress conditions in individual rats. *Physiol Behav* 45(5): 989-994. [http://dx.doi.org/10.1016/0031-9384\(89\)90226-6](http://dx.doi.org/10.1016/0031-9384(89)90226-6)
- De la Fuente J, Sánchez M, Pérez C, Lauzurica S, Vieira C, González de Chavarri E, Díaz MT, 2010. Physiological response and carcass and meat quality of suckling lambs in relation to transport time and stocking density during transport by road. *Animal* 4(2): 250-258. <http://dx.doi.org/10.1017/S1751731109991108>
- De la Fuente J, González de Chavarri E, Sánchez M, Vieira C, Lauzurica S, Díaz MT, Pérez C, 2012. The effects of journey duration and space allowance on the behavioural and biochemical measurements of stress responses in suckling lambs during transport to an abattoir. *Appl Anim Behav Sci* 142(1): 30-41. <http://dx.doi.org/10.1016/j.applanim.2012.08.010>
- Díaz MT, Vieira C, Pérez C, Lauzurica S, González de Chavarri E, Sánchez M, De la Fuente J, 2014. Effect of lairage time (0 h, 3 h, 6 h or 12 h) on glycogen content and meat quality parameters in suckling lambs. *Meat Sci* 96(2): 653-660. <http://dx.doi.org/10.1016/j.meatsci.2013.10.013>
- EC, 2004. Council Regulation No 1/2005 of 22 December. On the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97. *Official Journal of the European Union* L 44. <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32005R0001>
- EC, 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* L 276/33. <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010L0063>
- Ekiz B, Ekiz EE, Kocak O, Yalcintan H, 2012. Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. *Meat Sci* 90: 967-976. <http://dx.doi.org/10.1016/j.meatsci.2011.11.042>
- Gonyou HW, 2012. The welfare of sheep: review of recent literature. <http://www.prairieswine.com/wp-content/uploads/2012/02/sheep.pdf> [21.06.2015].
- Grandin T, 1997. Assessment of stress during handling and transport. *J Anim Sci* 75: 249-257.
- Grandin T, 2000. *Livestock handling and transport*, 2nd edn. CABI Publ., Wallingford, UK. 449 pp. <http://dx.doi.org/10.1079/9780851994093.0000>
- Gregory NG, Grandin T, 1998. *Animal welfare and meat science*. CABI Publ., Wallingford, UK. 298 pp.
- Gupta AK, Mamta YP, Yadav MP, 1999. Effect of feed deprivation on biochemical indices in equids. *J Equine Sci* 10(2): 33-38. <http://dx.doi.org/10.1294/jes.10.33>
- Hall SJG, Kirkpatrick SM, Lloyd DM, Broom DM, 1998. Noise and vehicular motion as potential stressors during the transport of sheep. *Anim Sci* 67(3): 467-473. <http://dx.doi.org/10.1017/S1357729800032884>
- Ibáñez M, De la Fuente J, Thos J, González de Chavarri E, 2002. Behavioural and physiological responses of suckling lambs to transport and lairage. *Anim Welf* 11(2): 223-230.
- IBM, 2010. *IBM SPSS Statistics for Windows (vers. 19.0)*. IBM Corp., Armonk, NY.
- Kannan G, Terrill TH, Kouakou B, Gazal OS, Gelaye S, Amoah EA, Samaké S, 2000. Transportation of goats: Effects on physiological stress responses and live weight loss. *J Anim Sci* 78(6): 1450-1457.
- Knowles TG, 1998. A review of the road transport of slaughter sheep. *Vet Rec* 143(8): 212-219. <http://dx.doi.org/10.1136/vr.143.8.212>
- Knowles TG, Warriss PD, Brown SN, Edwards JE, 1998. Effects of stocking density on lambs being transported by road. *Vet Rec* 142(19): 503-509. <http://dx.doi.org/10.1136/vr.142.19.503>
- Linares MB, Bórnez R, Vergara H, 2008. Cortisol and catecholamine levels in lambs: effects of slaughter weight and type of stunning. *Livest Sci* 115(1): 53-61. <http://dx.doi.org/10.1016/j.livsci.2007.06.010>
- Liotta L, Nanni Costa L, Chiofalo B, Ravarotto L, Chiofalo V, 2007. Effect of lairage duration on some blood constituents and beef quality in bulls after long journey. *Ital J Anim Sci* 6: 375-384. <http://dx.doi.org/10.4081/ijas.2007.375>
- Liste G, Miranda-de la Lama GC, Campo MM, Villarroel M, Muela E, María GA, 2011. Effect of lairage on lamb welfare and meat quality. *Anim Prod Sci* 51: 952-958. <http://dx.doi.org/10.1071/AN10274>
- Ljungberg D, Gebresenbet G, Aradom S, 2007. Logistics chain of animal transport and abattoir operations. *Biosyst Eng* 96(2): 267-277. <http://dx.doi.org/10.1016/j.biosystemseng.2006.11.003>

- Marco I, Lavín S, 1999. Effect of the method of capture on the haematology and blood chemistry of red deer (*Cervus elaphus*). *Res Vet Sci* 66(2): 81-84. <http://dx.doi.org/10.1053/rvsc.1998.0248>
- Mellor DJ, Stafford KJ, Todd SE, Lowe TE, Gregory NG, Bruce RA, Ward RN, 2002. A comparison of catecholamine and cortisol responses of young lambs and calves to painful husbandry procedures. *Aust Vet J* 80(4): 228-233. <http://dx.doi.org/10.1111/j.1751-0813.2002.tb10820.x>
- Miranda-de la Lama GC, Rivero L, Chacón G, García-Belenguer S, Villarroel M, María GA, 2010a. Effect of the pre-slaughter logistic chain on some indicators of welfare in lambs. *Livest Sci* 128(1): 52-59. <http://dx.doi.org/10.1016/j.livsci.2009.10.013>
- Miranda-de la Lama GC, Liste G, Villarroel M, Escós J, María GA, 2010b. Critical points in the pre-slaughter logistic chain of lambs in Spain that may compromise the animal's welfare. *Small Rumin Res* 90(1): 174-178. <http://dx.doi.org/10.1016/j.smallrumres.2010.02.011>
- Miranda-de la Lama GC, Monge P, Villarroel M, Olleta JL, García-Belenguer S, María GA, 2011. Effects of road type during transport on lamb welfare and meat quality in dry hot climates. *Trop Anim Health Prod* 43(5): 915-922. <http://dx.doi.org/10.1007/s11250-011-9783-7>
- Miranda-de la Lama GC, Salazar-Sotelo I, Pérez-Linares C, Figueroa-Saavedra F, Villarroel M, Sañudo C, María GA, 2012. Effects of two transport systems on lamb welfare and meat quality. *Meat Sci* 92(4): 554-561. <http://dx.doi.org/10.1016/j.meatsci.2012.05.026>
- Montané J, 2002. Valoración del estrés de captura, transporte y manejo en el corzo (*Capreolus capreolus*). Efecto de la acepromacina y de la cautividad. Doctoral thesis, Univ. Autónoma de Barcelona, Bellaterra, Spain.
- Pollard JC, Littlejohn RP, Asher GW, Pearse AJT, Stevenson-Barry JM, McGregor SK, Manley TR, Duncan SJ, Sutton CM, Pollock KL et al., 2002. A comparison of biochemical and meat quality variables in red deer (*Cervus elaphus*) following either slaughter at pasture or killing at deer slaughter plant. *Meat Sci* 60(1): 85-94. [http://dx.doi.org/10.1016/S0309-1740\(01\)00110-3](http://dx.doi.org/10.1016/S0309-1740(01)00110-3)
- Rabaste C, Faucitano L, Saucier L, Mormède P, Correa JA, Giguère A, Bergeron R, 2007. The effects of handling and group size on welfare of pigs in lairage and their influence on stomach weight, carcass microbial contamination and meat quality. *Can J Anim Sci* 87(1): 3-12. <http://dx.doi.org/10.4141/A06-041>
- Sañudo C, Sánchez A, Alfonso M, 1998. Small ruminant production systems and factors affecting lamb meat quality. *Meat Sci* 49(S1), S29-S64.
- SCAHAW, 2002. The welfare of animals during transport (details for horses, pigs, sheep and cattle). Scientific Committee on Animal Health and Animal Welfare, European Commission, Health and Consumer Protection Directorate-General. Report adopted on 11 March 2002. http://ec.europa.eu/food/fs/sc/scah/out71_en.pdf [14.06.2015].
- Tadich N, Gallo C, Brito M, Broom DM, 2009. Effects of weaning and 48 h transport by road and ferry on some blood indicators of welfare in lambs. *Livest Sci* 121(1): 132-136. <http://dx.doi.org/10.1016/j.livsci.2008.06.001>
- Tejeda JF, Peña RE, Andrés AI, 2008. Effect of live weight and sex on physico-chemical and sensorial characteristics of Merino lamb meat. *Meat Sci* 80(4):1061-1067. <http://dx.doi.org/10.1016/j.meatsci.2008.04.026>
- Zimmerman M, Grigioni G, Taddeo H, Domingo E, 2011. Physiological stress responses and meat quality traits of kids subjected to different pre-slaughter stressors. *Small Rumin Res* 100(2): 137-142. <http://dx.doi.org/10.1016/j.smallrumres.2011.06.011>
- Zimmerman M, Domingo E, Grigioni G, Taddeo H, Willems P, 2013. The effect of pre-slaughter stressors on physiological indicators and meat quality traits on Merino lambs. *Small Rumin Res* 111(1): 6-9. <http://dx.doi.org/10.1016/j.smallrumres.2012.12.018>