CSIRO PUBLISHING

Animal Production Science, 2014, **54**, 179–186
http://dx.doi.org/10.1071/AN13024

Effect of the duration of road transport on the physiology and meat quality of lambs

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Abstract. To assess the effect of transport duration on animal welfare and meat quality of lambs, two trials were performed: Forty Ile-de-France × Merino lambs were used in Trial 1 and 40 Comisana lambs in Trial 2. In both trials, the lambs, aged between 14 and 16 weeks, were divided into two groups of 20 animals. One group was subjected to a 1-h period of transportation (T1) and the other to a 24-h period of transportation (T24), both in the same truck and arriving to the same slaughterhouse at the same time. The effect of transport on serum biochemistry variables (cortisol, aspartate transaminase, lactate dehydrogenase, blood urea nitrogen, creatine kinase, creatinine and total proteins), salivary cortisol, metabolites of cortisol in faeces, intra-ruminal temperature and meat quality (pH, conductivity, expressible juice, colour and shear force) was assessed. In both studies, the duration of transport did not affect serum and salivary cortisol concentration ($P > 0.05$). However, in Trial 2, lambs exposed to 24-h transport had a higher concentration of faecal cortisol metabolites than did those transported for 1 h ($P < 0.05$). Blood variables were not affected by transport in either trial ($P > 0.05$), with the exception of blood urea nitrogen which was higher in Trial 1 for the T24 group ($P < 0.05$) than it was to T1 group. Although no signs of dehydration were found, intra-ruminal loggers showed that animals did not drink during the transportation in the way they did before transport. In Trial 1, no effect of transport duration was found on meat-quality traits ($P > 0.05$). Nevertheless, in Trial 2, lambs exposed to 24-h transport had higher values of colour attribute of a* (red trend) and less tenderness or higher values of shear force ($P < 0.05$). The present study showed that although there is little effect on meat quality, signs of stress are detectable in lambs transported for 24 h. Therefore, in the case of lambs, the effect of long transportation periods must be considered more in terms of animal welfare than in terms of product quality.

Additional keywords: animal welfare, cortisol.

Received 22 January 2013, accepted 26 March 2013, published online 15 May 2013

Introduction

Commercial transport of livestock for slaughter is unavoidable. The progressive reduction in the number of small abattoirs and the construction of big industrial slaughterhouses collecting animals from large areas results in animals being transported over larger distances for slaughter. Moreover, the ease of animal trade among countries, facilitated by international agreements (e.g. EU free-trade), by the improvement of road networks and in the technical characteristics of vehicles, has led to an increase in the road transport of animals among European countries. These factors result in an increased transportation distance, raising the problem of possible negative consequences on animal welfare and meat quality in pigs (Bradshaw *et al*. 1996; Pérez *et al*. 2002) and lambs (Cunha Leme *et al*. 2012).

Growing societal interest in animal welfare has been reported (Velarde and Dalmau 2012), and has originated from the acknowledgement that animals are sentient beings and that any act that would increase their suffering is considered morally unacceptable. In the past few years, this has become a worldwide issue (Fraser 2008) and laws concerning animal welfare have been established in many countries.

Welfare has been defined as the state of an individual with regard to its attempts to cope with its environment (Broom 1986). Broom (1988) defined stress as an environmental effect on an individual animal that over-taxes the animal's control systems and reduces its fitness, thus impairing welfare. Stress is an unavoidable consequence of the process of transferring animals from farm to abattoir (Ferguson and Warner 2008) and road transport-related stress could have a great influence on animal performance, increasing mortality and lowering meat quality, with heavy economic implications (Swanson and Morrow-Tesch 2001).

Pre-slaughter handling affects muscle glycogen metabolism, leading to abnormal muscle acidification, and may increase the incidence of dry, firm, dark (DFD) meat (Gregory 1998; Adzitey and Nurul 2011). Anomalous acidification of muscular tissue also makes meat more susceptible to microbiological alterations, with repercussions on food safety (Seidler *et al*. 2001). Previous research on small ruminants studied the connection between carcass lesions (haematomas, fractures, ecchymosis) and pre-slaughter transport (Cockram and Lee 1991; Jarvis and Cockram 1994; Jarvis *et al*. 1996; Knowles 1998), and investigated the economic impact of these factors (Speer *et al*. 2001). The main goal of the present study was to evaluate the effects of the duration of pre-slaughter transport on lamb welfare and meat quality. To carry out the study, two different trials were performed in different seasons with lambs of similar ages but different breeds, due to the market conditions when the trials were performed.

Materials and methods

Animals

In Trial 1, 40 Ile-de-France × Merino lambs (24 males and 16 females) aged 14–16 weeks and ~17 kg liveweight were used. In Trial 2, 40 Comisana lambs (32 males and 8 females) of the same age and ~13 kg liveweight were used. All lambs were fed with ad libitum hay (clover and oat) and concentrated (corn, peas and barley). Animals from both trials came from the same farm, located in Ruvo di Puglia (41°7′0″N, 16°29′0″E), in the south-east of Italy.

Procedure

Trial 1 was carried out in March and Trial 2 in November 2009. Two days before the study, 40 animals were selected for each trial and housed in a separate pen on the farm. Thirty-six hours (Trial 1) and 60 h (Trial 2) before the beginning of the transportation period, 20 animals were assigned to each of the two treatments in a random way (transported for 24 h (T24) and transported for 1 h (T1)). Although all the lambs were housed in the same pen, T24 and T1 animals were marked with different colours to distinguish the two groups. At the same time, while marking the animals, a sample of blood was collected from the right jugular vein with an 18-gauge needle for determination of cortisol, creatine kinase (CK), total protein (TP), lactate dehydrogenase (LDH), aspartate transaminase (AST), creatinine and blood urea nitrogen (BUN) in two vacutainer tubes (Precision glide, BD, Franklin Lakes, NJ, USA) at 36 h (Trial 1) and 60 h (Trial 2) before the long transportation period. A sample of faeces from the rectum and, only for Trial 2, a sample of saliva (using Salivette cortisol system, Starstedt, Verona, Italy) were also taken at the same time to assess cortisol metabolites and cortisol concentrations, respectively. Following this, an intra-ruminal logger (ThermoChron, IDC, Barcelona, Spain) registering temperature was given by oral administration to eight animals in each treatment. The logger recorded the temperature in °C every 5 min from 36 h (Trial 1) and 60 h (Trial 2) before the long transportation period to the slaughterhouse; the truck used for the study was the same for both trials and its dimensions were 13.30 m length, 2.55 m width and 0.90 m height and it had three floors. In the present study, only the second and third compartments of the first floor of the truck were used. Both compartments had the same dimensions (3.25 m length by 2.55 m width; 0.4 m^2 per animal). In both trials, animals from T24 group were allocated in the second compartment and lambs from T1 group were allocated in the third compartment. The T24 group was loaded at 0620 hours in Trial 1 and at 0645 hours in Trial 2. In Trial 1, the T24 transport consisted of a 1578-km trip from south to north and return to the south of Italy, with a total of 18 hours and 40 minutes on the road and 4 hours and 20 minutes stationary. The mean speed was 79.91 km/h, temperature ranged from 2°C (at 0420 hours on Day 2) to 16°C (at 1300 hours on Day 1). The weather was sunny or partly cloudy and it rained from 2330 hours to 0215 hours. In Trial 2, the T24 transport was the same as in Trial 1. The mean speed was 83 km/h, temperature ranged from 7°C (at 0830 hours on Day 1) to 17°C (at 1100 hours on Day 1). The weather was partly cloudy but it did not rain during the T24 transport. The truck returned to the farm of origin at 0530 hours in Trial 1 and at 0710 hours in Trial 2. After a stop, the second group of animals (T1) was loaded in the third compartment of the first floor of the truck and the short transport began (at 0750 hours in Trial 1 and at 0830 hours in Trial 2). Then, both groups of animals were transported to a slaughterhouse situated 42 km away from the farm. Water was available to animals during the whole study by means of troughs in the farm and lairage pens of the slaughterhouse and by nipples on the truck.

In both trials, it rained during the shorter transport for 15 min in Trial 1 and during the whole trip in Trial 2. Animals were unloaded at 0905 hours in Trial 1 and at 0940 hours in Trial 2. Both T24 and T1 groups were allocated in the same lairage pen where, immediately after arrival, blood and faecal samples were taken. In Trial 2, a second sample of saliva was also taken at this point of time. The 40 lambs were slaughtered randomly ~1200 hours in Trial 1 and ~1300 hours in Trial 2. After the evisceration, temperature loggers were recovered from rumen or reticulum. In both trials, carcass- and meat-quality measurements were performed 24 h after slaughter. Immediately after slaughter, carcasses were chilled and maintained at 4°C.

Carcass- and meat-quality measurements

Cold-carcass weight was recorded 24 h post mortem and the left- and right-side loins were removed from each carcass from the 6th thoracic rib to the 7th lumbar vertebra. Left-side loins were vacuum packaged, kept in refrigeration (2°C), and frozen after 72 h (−20°C) for subsequent instrumental tenderness determination by Warner–Bratzler shear force (WBSF).

Muscle pH was measured at 24 h post mortem on the right-side loin, at the 6th rib level of *longissimus thoracis* (LT), using a Crison portable 159 m (Crison, Barcelona, Spain) equipped with a xerolyt electrode. After measuring pH, a 6 cm loin section was removed from the 6th rib level towards the caudal end of the loin for expressible moisture determination. Instrumental colour measurements were recorded for lightness (L*), redness to greenness (a*), and yellowness to blueness (b*) on the exposed cut surface of the LT muscle after 30 min of bloom time, using a Minolta Chroma Meter (CR-400, Minolta Inc., Osaka, Japan) in the CIELAB space with Illuminant D65 and 2° viewing angle.

Expressed juice was assessed by using a filter paper press method (Whatman No. 1, 11.0 cm in diameter), with a meat sample weighing 0.5 g and a 2.25 kg weight applied to the sample between plexiglas plates for 5 min (Hamm 1986). Expressed juice was calculated by difference between the initial and final sample weights, and expressed as a percentage of the initial sample weight.

After being transported to the laboratory (IRTA–Monells, Spain) and frozen at −20°C for 15 days, loins were thawed for 24 h at 2°C and cooked in a convection oven (Spider 5, Novosir, Spain) pre-heated to 200°C, to an endpoint temperature of 71°C. Internal temperature was monitored using a thermocouple with a data logger, inserted into the geometric centre of the loin. Loins were left to come to room temperature and six 1.27 cm diameter cores were removed parallel to the longitudinal orientation of the muscle fibres from the middle portion of each cooked LT. All cores were sheared perpendicular to the long axis of the core by using a texture analyser (Alliance RT/5 MTS Systems Corporation, Eden Prairie, Minneapolis, USA) equipped with a Warner–Bratzler blade. Results are expressed in kilograms.

Clinical measures

Two blood-samples were taken from each animal, one for cortisol analysis and the other for assessment of blood biochemistry parameters (CK, TP, LDH, AST, creatinine and BUN).

The blood samples for cortisol analysis were immediately centrifuged (both on the farm and at the slaughterhouse) at 1000*g* for 15 min and serum was frozen at −5°C for 72 h and then stored at −20°C until it was analysed. The saliva samples for cortisol were also immediately centrifuged at 2000*g* for 10 min, frozen to −5°C for 72 h and then stored at −20°C until further analyses. In both cases, cortisol was determined by means of a competitive ELISA. The reagent used was salivary cortisol ELISA, SLV-2930 (DRG Diagnostics, Marburg, Germany) and the plates were analysed with EMS Reader MF V.2.9-0 (Labsystems, Helsinki, Finland). The results are expressed as nanograms of cortisol per millilitre.

The faecal samples taken for assessing cortisol metabolites were frozen immediately at −5°C for 72 h and then stored at −20°C until they were analysed. The extraction procedure was performed by adding 5 mL of methanol (80%) on 0.5 g portions of wet faeces. After shaking for 1 min on a hand-vortex and 45–60 minutes on the orbital shaker, followed by centrifugation at 1500*g* for 15 min, the extracted faecal samples were diluted 1 : 10 in phosphate-buffered saline (pH = 7.4) and analysed for cortisol metabolites according to Morrow *et al*. (2002), using the commercially available I^{125} radioimmunoassay kit (Rats & Mice ImmuChem™ Double Antibody, ICN Biomedicals Inc. Diagnostic Division, Solon, OH, USA). Results are expressed as nanograms of metabolite per gram of dry faeces.

Immediately after the extraction, the blood samples for assessing CK, TP, LDH, AST, creatinine and BUN were transported to the laboratory and processed. Sample analysis was performed with an enzymatic and colorimetric diagnostic kit (Instrumentation Laboratory Spa, Ascoli Piceno, Italy) using an ILAB 650 automated system (Instrumentation Laboratory Spa). All tests were performed following kit instructions. Preciseness of methods and instrumentation agreed the SIBIOC criteria (Schumann *et al.* 2002). Results are expressed in international units per litre for CK, AST and LDH, milligrams per decilitre for BUN and grams per decilitre for TP.

Statistical analyses

Each trial was analysed separately. Data from serum, salivary and faecal cortisol, in addition to blood biochemistry measures (CK, TP, LDH, AST, creatinine, BUN and creatinine : BUN ratio) and ruminal temperatures were analysed using the PROC MIXED procedure of SAS (SAS 9.2 Institute Inc., Cary, NC, USA) for repeated measures (Littell *et al.* 1996). The models accounted for the effects of duration of transport (long and short), time of the sample extraction (basal or after transport) and the interaction of duration of transport × time of sample. The different variables were subjected to the following three covariance structures: compound symmetric, autoregressive order one and unstructured covariance. The covariance structure that yielded the smallest Schwarz's Bayesian criterion and was closest by the likelihood value was considered to be the most suitable analysis. Unstructured covariance was applied to all the variables except for ruminal temperature, where compound symmetric was used. The residual maximum likelihood was used as a method of estimation. The least square means of fixed effects adjusted to Tukey's honestly significant difference were used to carry out multiple comparisons. Meat-quality variables (pH 24 h, L*, a* and b*, water-holding capacity and WBSF) were analysed by using the general linear model procedure of SAS. The model took into account the duration of transport, animal gender (ewe vs ram lambs) and the interaction between the duration of transport and animal gender. As differences were found among treatments in cold-carcass weight, this variable was included as a covariate in the models of meat-quality variables. There were no interactions ($P > 0.05$) between the duration of transport and gender for meat-quality characteristics and data are presented as main effects. Significance was fixed at $P < 0.05$ in all cases.

Results

Physiological response

In Trial 2, mean serum cortisol concentration was higher after transport than before transport ($P < 0.001$), although no interaction was found between the duration of transport and time of sample (Table 1). However, in Trial 1, no significant differences were found between values obtained before and after transport in either treatment. In Trial 1, faecal cortisol metabolites showed a higher mean concentration after transport than they did before in both treatments ($P < 0.001$). In Trial 2, it was observed that the concentration of faecal cortisol metabolites was higher after than before transport only for the T24 group ($P < 0.001$). In addition, the concentration of faecal cortisol metabolites after the transport was higher in animals from T24 than in those from T1 ($P < 0.001$). In the case of salivary cortisol, which was determined only in Trial 2, a very similar result was obtained, but the *P*-value for the interaction treatment by sample was indicative a trend only ($P = 0.0706$). In both trials, a different ruminal temperature was found between the T24 and T1 group

across the whole study, including the period before transport (Fig. 1, Table 2). However, although the ruminal temperature in T1 was similar throughout the study, in T24, it was lower ($P < 0.001$) while animals were being transported than it was before transport.

Biochemical parameters

No differences between before and after transport samples in T1 and T24 were found for CK, TP and AST in Trials 1 and 2, for LDH in Trial 1 and for creatinine in Trial 2 (Table 3). The mean values of creatinine in Trial 1 and LDH, BUN and BUN : creatinine ratio in Trial 2 were different before and after the transport ($P = 0.0130$, $P = 0.0350$, $P < 0.001$ and $P < 0.001$, respectively), but no effect of the duration of transport was found ($P > 0.05$). Finally, T24 animals in Trial 1 showed higher concentrations of BUN in samples taken after the transport ($P < 0.0001$) than in those taken before and those observed in T1 ($P < 0.0001$). The same pattern was observed for the BUN : creatinine ratio in Trial 1 ($P < 0.0001$).

Meat quality

Although there were no significant ($P > 0.05$) differences in carcass weight between the transport treatments (Tables 4 and 5), the weights of carcasses from lambs exposed to T24 were

Table 1. Least square means ± s.e. of physiological measures (serum and salivary cortisol and faecal metabolites of cortisol) in lambs subjected to a transport of 24 h (T24, $n = 20$) or 1 h (T1, $n = 20$) for samples taken before or after the transport in Trials 1 and 2

Means within the same column followed by a different lower-case letter are significantly different ($P < 0.01$). Means within the same row within each trial followed by a different upper-case letter are significantly different ($P < 0.01$)

Variable	Duration	Trial 1		Trial 2	
		Before transport	After transport	Before transport	After transport
Serum cortisol (ng/mL)	T24	23.8 ± 3.52	19.2 ± 3.52	10.1 ± 2.36X	17.6 ± 2.37Y
	T1	23.6 ± 3.52	21.2 ± 3.52	10.6 ± 2.43X	17.3 ± 2.37Y
Faecal cortisol metabolites (ng/g DM)	T24	33.3 ± 8.78X	81.6 ± 8.77Y	36.8 ± 6.49X	83.1 ± 6.89aY
	T1	33.9 ± 9.02X	69.8 ± 8.55Y	33.8 ± 6.49	39.8 ± 6.49b
Salivary cortisol (ng/mL)	T24	–	–	1.61 ± 0.706X	6.31 ± 0.651Y
	T1	–	–	1.70 ± 0.706X	3.90 ± 0.651Y

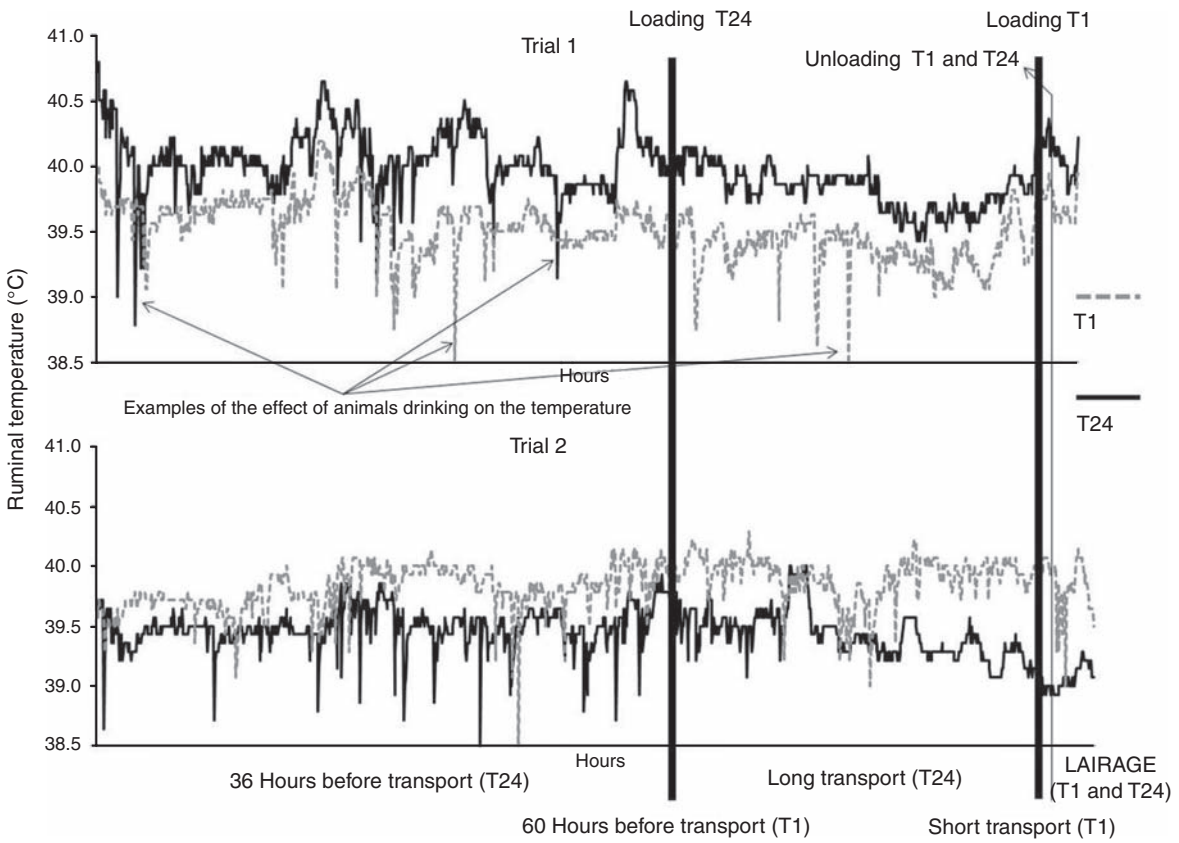

Fig. 1. Mean values of ruminal temperature taken at 5-min intervals for lambs transported for 24 h (T24) and 1 h (T1) in Trial 1 and Trial 2 ($n = 8$ for each treatment). Temperature before, during transportation periods and thereafter until slaughter is shown.

lower than those of animals exposed to T1 in both trials (0.21 and 0.42 kg lighter for Trials 1 and 2, respectively). In Trial 1, there was no effect ($P > 0.05$) of the duration of transport or gender on meat-quality characteristics, except for colour L* which was significantly ($P = 0.0062$) higher for rams than for ewe lambs. In Trial 2, carcasses from rams were ~1.2 kg heavier ($P = 0.0003$) than carcasses from ewe lambs, but there was no effect ($P > 0.05$) of gender on meat-quality characteristics. Long transportation resulted in redder ($P = 0.0469$) and tougher meat ($P = 0.0259$) than did short transportation. Transport duration did not affect ($P > 0.05$) pH, L* and b*, or expressible juice of meat from transported lambs.

Discussion

There was no effect on serum cortisol as a result of transport duration (both trials) nor from before and after transportation (Trial 1 only). This result is in accordance with Fisher *et al*. (2010) who found no effect on serum cortisol for transportation durations of 12 h, 30 h and 48 h in mature Merino ewes. According to these authors, this was due to sheep adaptation to the transport conditions during the journey. Other researchers have stated that serum cortisol concentration increases in sheep at the beginning of the journey, after loading, and decreases to more basal values over a few hours (Broom *et al*. 1996; Cockram *et al*. 1997). Therefore, an increase in serum cortisol in the T1 group would have been expected even in the present study, but this was not the case. Two possible explanations are proposed. The first one is that transportation for 1 h may not have been stressful enough to elicit an increase in circulating cortisol in these lambs. The second is that, although the blood sample was taken in less than 1 min per animal, similar to Fisher *et al*. (2010), the disturbance produced by management procedures may have induced a stress response that interfered with the cortisol results. In contrast, in Trial 2, an increase in serum cortisol was detected after transport in relation to values obtained before transport in both treatments. Weather conditions differed between the two trials; in Trial 2, it rained throughout the period of the short transportation, whereas the conditions before transport where not different between trials (same farm and similar temperatures). In addition, Hall and Bradshaw (1998) suggested that there is a breed difference, which in their case was Clun Forest and Suffolk × Greyface, and this could explain the differences found in the susceptibility to transportation in sheep among different studies. Therefore, an effect of breed cannot be excluded in the present study. In Trial 2, in addition to blood samples, salivary samples were taken for cortisol assessment to provide more reliable information on acute stress. In fact, in plasma, most cortisol is protein bound and only free cortisol acts in the body. Cortisol reaches saliva by diffusion in salivary gland cells and its diffusion rate is high enough to maintain an equilibrium between the free cortisol in plasma and saliva (Broom 2000). Any rise in the concentration

Table 2. Least square means ± s.e. of ruminal temperature (°C) in lambs subjected to a transport of 24 h (T24, $n = 20$) or 1 h (T1, $n = 20$) for samples taken before the transport, while the animals from the T24 were being transported (from 0 to 23 h) and while the animals from both treatments were being transported (from 23 to 24 h) in Trials 1 and 2

Means within the same column within each trial followed by a different lower-case letter are significantly different ($P < 0.01$). Means within the same row followed by a different upper-case letter are significantly different ($P < 0.01$)

Parameter	T24	T1
	Trial 1	
Before transport	39.95 ± 0.010aX	39.55 ± 0.011aY
0 to 23 h	39.74 ± 0.010bX	39.41 ± 0.011bY
23 to 24 h	39.75 ± 0.018bX	39.57 ± 0.019aY
	Trial 2	
Before transport	39.51 ± 0.011aY	39.89 ± 0.011X
0 to 23 h	39.39 ± 0.021bY	39.88 ± 0.011X
23 to 24 h	39.17 ± 0.021cY	39.84 ± 0.021X

Table 3. Least square means ± s.e. of creatine kinase (CK), aspartate transaminase (AST), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), total protein (TP) and creatinine in serum of lambs subjected to a transport of 24 h (T24, $n = 20$) or 1 h (T1, $n = 20$) for samples taken before or after the transport in Trials 1 and 2

Means within the same column for each metabolite followed by a different lower-case letter are significantly different ($P < 0.01$). Means within the same row within each trial followed by a different upper-case letter are significantly different ($P < 0.01$)

Metabolite	Treatment	Trial 1		Trial 2	
		Before transport	After transport	Before transport	After transport
CK (IU/L)	T24	3594 ± 1123.8	6216 ± 1123.8	355 ± 385.0	350 ± 385.0
	T1	1267 ± 1123.8	906 ± 1123.8	1251 ± 385.0	216 ± 385.0
AST (IU/L)	T24	502 ± 140.4	894 ± 140.4	99 ± 36.1	81 ± 36.1
	T1	325 ± 140.4	328 ± 140.4	183 ± 36.1	113 ± 36.1
LDH (IU/L)	T24	2330 ± 414.2	3292 ± 414.2	1089 ± 125.5Y	925 ± 125.5X
	T1	1338 ± 414.2	1374 ± 414.2	1380 ± 125.5Y	1004 ± 125.5X
BUN (mg/dL)	T24	10.00 ± 1.047X	18.00 ± 1.047aY	8.45 ± 0.958X	16.05 ± 0.958Y
	T1	9.95 ± 1.047	8.25 ± 1.047b	9.05 ± 0.958X	13.85 ± 0.958Y
TP (g/dL)	T24	5.56 ± 0.093	5.62 ± 0.093	6.17 ± 0.073	6.12 ± 0.073
	T1	5.64 ± 0.093	5.62 ± 0.093	6.04 ± 0.073	6.02 ± 0.073
Creatinine (mg/dL)	T24	0.89 ± 0.022Y	0.81 ± 0.022X	0.90 ± 0.022	0.92 ± 0.021
	T1	0.86 ± 0.022Y	0.83 ± 0.022X	0.89 ± 0.022	0.93 ± 0.021
BUN : creatinine	T24	11.2 ± 1.30X	22.6 ± 1.30aY	9.5 ± 1.07X	17.4 ± 1.07Y
	T1	11.6 ± 1.30	9.8 ± 1.30b	10.2 ± 1.07X	15.2 ± 1.07Y

Table 4. Least square means ± s.e. of carcass weight and meat quality of ewe and ram lambs of meat breed transported for 24 h (T24) and for 1 h (T1) in Trial 1 (n = 40)

WBSF = Warner–Bratzler shear force. Means within a row for each comparison category followed by a different letter are significantly different ($P < 0.01$)

Carcass and meat quality	Transport		Gender	
	T1 (n = 20)	T24 (n = 20)	Ewes (n = 16)	Rams (n = 24)
Cold-carcass weight (kg)	7.60 ± 0.396	7.39 ± 0.396	7.67 ± 0.434	7.32 ± 0.355
pH 24 h	5.66 ± 0.071	5.72 ± 0.071	5.65 ± 0.078	5.73 ± 0.064
L* lightness	43.2 ± 0.71	44.4 ± 0.71	42.3b ± 0.78	45.3a ± 0.64
a* redness	12.7 ± 0.36	12.6 ± 0.36	13.1 ± 0.39	12.3 ± 0.32
b* yellowness	2.76 ± 0.223	3.27 ± 0.223	2.72 ± 0.244	3.31 ± 0.199
Expressed juice (%)	18.3 ± 0.54	18.5 ± 0.54	17.9 ± 0.59	18.9 ± 0.48
WBSF (kg)	5.45 ± 0.420	5.61 ± 0.420	5.76 ± 0.461	5.30 ± 0.376

Table 5. Least square means ± s.e. of carcass weight and meat quality of ewe and ram lambs of dairy breed transported for 24 h (T24) and 1 h (T1) in Trial 2 (n = 40)

WBSF = Warner–Bratzler shear force. Means within a row followed by a different letter are significantly different ($P < 0.01$)

Carcass and meat quality	Transport		Gender	
	T1 (n = 20)	T24 (n = 20)	Ewes (n = 8)	Rams (n = 32)
Cold-carcass weight (kg)	5.42 ± 0.182	5.00 ± 0.221	4.63b ± 0.258	5.79a ± 0.125
pH 24 h	5.77 ± 0.018	5.79 ± 0.024	5.78 ± 0.030	5.78 ± 0.013
L* lightness	41.9 ± 0.86	40.44 ± 1.12	40.2 ± 1.41	42.1 ± 0.61
a* redness	12.5b ± 0.35	13.6a ± 0.46	13.4 ± 0.58	12.7 ± 0.25
b* yellowness	2.31 ± 0.234	2.57 ± 0.305	2.39 ± 0.382	2.48 ± 0.166
Expressed juice (%)	21.0 ± 1.37	21.1 ± 1.79	19.8 ± 2.25	22.3 ± 0.98
WBSF (kg)	7.14b ± 0.355	8.46a ± 0.463	8.13 ± 0.580	7.46 ± 0.252

of salivary cortisol following a stimulus is delayed by a few minutes, as compared with the corresponding increase in plasma cortisol concentration (Broom 2000). This provides the advantage of producing a result that is not biased by the blood sampling procedure. According to Fell *et al*. (1985), the concentration of salivary cortisol is 1/10, or less, of the concentration in plasma. In the present study, salivary cortisol was one-third to one-sixth of the serum cortisol. Again, the mean concentration of salivary cortisol was found to be significantly ($P < 0.001$) higher after than before transportation, with a statistical trend ($P = 0.0943$) obvious for the comparison between the T24 (6.3 ng/mL) and T1 (3.9 ng/mL) groups. Faecal cortisol metabolites were also determined. A difference was observed in Trial 1 in the sample taken 61 h after the basal sample, without significant differences between T24 and T1. One possible explanation for the lack of differences between the two treatments could be a lack of time for animals to recover their basal cortisol concentrations in faeces after the management intervention on Day 0 (blood and faecal sampling and temperature-logger allocation) in Trial 1. In Trial 2, the resting time was increased of 24 h. As a result, in Trial 2, faecal cortisol concentrations were higher after transport than was the basal concentration in T24 group, but not in T1. Therefore, the similar serum cortisol concentration, the possible higher salivary cortisol concentration and the higher faecal cortisol concentration in animals transported for 24 h than in those transported for 1 h would suggest that an accumulative stress exists in lambs transported for long periods.

Animals having substantial adrenal cortex responses during handling and transport show increased body temperature (Trunkfield and Broom 1990). In our study, a thermal data logger was used to monitor the internal temperature of animals. The results showed that T24 animals had a lower ruminal temperature (~0.2°C lower) during transportation than they did before departure, whereas T1 animals maintained similar values during the whole period in both trials. A possible explanation for this slight reduction of the ruminal temperature could be the food deprivation that animals undergo during transport. The allocation of the loggers in rumen allowed us to observe the drinking patterns of the animals. In fact, when animals drank water (the temperature of which was always lower than the internal temperature of the animal), intra-ruminal temperature showed a very significant decrease. In Fig. 1, it can be seen that in both trials, animals in T24 group did not have these falls in temperature after being loaded in the truck, even though the truck had drinkers. It is important to state that values shown in Fig. 1 are mean values per treatment in which these falls can be clearly identified, but when data are treated individually (8 animals per treatment were assessed), the falls are most obvious. There is also additional evidence that animals did not drink in the truck as they did on the farm. In addition, it can be seen that in Trial 2, two of the eight animals of the T1 group with loggers drank water after arriving at the lairage pens, which was not the case for the eight monitored animals from the T24 in this trial nor for any animals with loggers (16) in Trial 1. This fact had been noticed also by the observers at

the slaughterhouse. Therefore, although some signs of thirst could be seen, especially in Trial 1, where animals were observed licking the rain water from the bars of the truck during some of the stops, they did not use the drinkers at the abattoir. However, no significant changes in TP in serum were reported in either trial, thus suggesting that animals were not seriously dehydrated. The weather conditions during the study, with maximum temperatures of 16°C and 17°C in Trials 1 and 2, respectively, do not allow to us to determine whether higher temperatures would induce clearer signs of dehydration in the animals or just higher motivation to find and use the drinkers in the truck and lairage pens. However, this should be considered an important subject for further research. For instance, Jacob *et al*. (2006), studying urine samples collected from lambs at the slaughterhouse over a year, found signs of subclinical dehydration very commonly in these animals.

BUN and serum creatinine can increase during stress situations because of the increased muscular activity, due to the action of catecholamines (Bórnez *et al*. 2009) or due to an increase in protein catabolism, as occurs in the case of long periods of food deprivation (Knowles 1998). No changes in creatinine concentration were observed during the present study, but an increase in BUN in Trial 1 was found as a result of transport in T24 group and between T24 and T1 after transport, being higher in T24. These changes could be attributable to food deprivation, more pronounced in T24 because of the increased duration of transport as compared with T1. In addition, since BUN concentration can be linked to the glomerular filtration rate (Russell and Roussel 2007), the deficiency in water intake might have contributed to these findings.

No major differences were found in CK, LDH and AST in either trial between the T24 and T1 groups. In domestic ungulates, these enzymes are known to be the most sensitive detectors of muscular alterations during stress (Marco *et al*. 1998) and indicators of high muscular activity, muscular damage, tissue damage or muscular exertion (Bórnez *et al*. 2009). The lack of statistical differences in the activity of these enzymes demonstrated that no trauma occurred during the study.

Animal stress associated with transport can have a significant deleterious effect on lamb meat quality (Ferguson and Warner 2008). There is scientific evidence that sheep, compared with other farmed species, are particularly tolerant of being transported (Knowles 1998).

In Trial 2, meat from animals transported for 24 h was redder and showed an 18.5% increase in shear force (1.32 kg higher) compared with meat from lambs transported for 1 h. Many trials have shown higher shear forces and lower tenderness in stressed lambs, which could be associated with reduced glycogen concentrations resulting in high pH values (>6.1–6.2, Miranda-de la Lama *et al*. 2011). However, in the present study, pH was unaffected by the experimental procedures, with pH remaining within an acceptable range for the meat market, with no evidence of dark cutting. Miranda-de la Lama *et al*. (2011) reported that lambs transported on unpaved roads had a more intense stress response, higher pH and tougher meat, but similar water-holding capacity, than did lambs transported on paved roads. Further research is needed in this area, to better understand the relationship between stress induced by animal handling before slaughter, meat tenderness and other meat-quality parameters such as muscle pH and water-holding capacity.

Conclusions

According to the concentration of faecal cortisol metabolite in animals transported for 24 h in relation to those transported for 1 h, an accumulative stress response could exist in lambs transported for long periods that was not detectable in the blood samples. According to the intra-ruminal temperature, animals did not drink, at least in the way they did before transport, during the transport and, in the case of T24, in lairage pens. No meat-quality effects were found in Ile-de-France × Merino lambs, but in Comisana breed, an effect on the colour attribute a* (red trend) and tenderness or maximum shear force was observed, being worse for animals transported for 24 h than for those transported for 1 h. Therefore, although lambs transported for 24 h have to cope with challenges that can affect their welfare, few effects on meat quality were found in comparison to a transportation period of 1 h. In consequence, in the case of lambs, the effect of long transportation periods must be considered more in terms of animal welfare than in terms of product quality.

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