Serum stress parameters in pigs transported to slaughter under commercial conditions in different seasons

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ABSTRACT: To determine the influence of slaughter transports carried out under commercial conditions, 162 pigs weighing 98 kg and of both sexes were studied. A total of seven transports were performed in summer and in winter conditions, with durations of 1 h and 13 h 15 min within each season. Cortisol, glucose, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), albumin and total protein serum concentrations were measured. All variables increased during transport and decreased during lairage ($P < 0.001$), with cortisol values being 3.47 ± 0.19, 8.52 ± 0.28, and 6.96 ± 0.18 μg/dl at loading, unloading and exsanguinations, respectively, except for glucose (0.54 ± 0.03, 0.44 ± 0.04, and 0.86 ± 0.03 g/l). Short journeys did not allow the total recovery from the loading stress. A certain level of dehydration was observed, especially during lairage on the longest journeys (increase of 6.87 ± 1.29 g/l for total proteins; $P < 0.01$). Winter transports were slightly more stressful ($P < 0.01$ for cortisol and LDH, and $P < 0.001$ for CPK), with poorer recovery during lairage (CPK decrease being –141 ± 559 and –2 906 ± 730 IU/l for winter and summer journeys, respectively; $P < 0.01$). Females showed higher stress reactivity. Genetics modulated the effect of the rest of influencing factors, with Nn individuals showing a rougher reaction in short and winter conditions, but with lower dehydration levels. Under Mediterranean commercial conditions, stress in transported slaughter pigs was largely determined by season and genetics, so that an adaptation of handling procedures to these seasonal variations appears crucial if transport stress is to be reduced. Also, an improvement in stress resistance could be obtained by controlling the halothane gene of pigs.

Keywords: halothane gene; pig; season; stress; transport duration; welfare

The transport of slaughter pigs is a vitally important issue in swine production, involving all the productive agents of the sector and implying important economic connotations, with almost 38 million pigs being slaughtered in Spain during 2005 (Eurostat). Nevertheless, transport and the associated handling may cause important losses to the livestock industry (Grandin, 2000), and social concern about the consequences of transport on animal welfare has gradually increased during recent decades, resulting in a legislation being past in the European Union (Council Regulation 1/2005/EC).

An extensive bibliography about the effect of transport on the stress of slaughter pigs already exists (see review by Warriss, 1998). Among other factors, it is known that the duration of the journey has a negative influence on their welfare and their meat quality (Bradshaw et al., 1996; Fabrega et al., 2002; Perez et al., 2002; Mota-Rojas et al., 2006) although, if proper handling is provided before and throughout transportation, stress levels may

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be reduced (Gosalvez et al., 2006) even during long journeys (Brown et al., 1999a).

The effect of climatic conditions during pigs transport has also been studied, although with conflicting results. Some authors found that cold and heat stress may have a negative effect on meat quality (O'Neill et al., 2003; Guardia et al., 2004, 2005; dalla Costa et al., 2006), and that high temperatures increase mortality during transport (Smith and Allen, 1976; Abbott et al., 1995; Vecerek et al., 2006), although other researchers did not find the highest mortalities during the hottest months (Palacio et al., 1996; Gosalvez et al., 2006), with a higher proportion of skin bruises being reported in winter (dalla Costa et al., 2006). Nevertheless, to our knowledge, little or no information exists about the effect of seasonal transportation on the blood parameters of slaughter pigs.

Additionally, a genetic predisposition may aggravate the negative impact of transport, since it is well documented that halothane gene carrier pigs are more responsive to a stressful situation such as transportation (Rundgren et al., 1990; Geers et al., 1994), with their welfare and meat quality being impaired (Gispert et al., 2000; Fabrega et al., 2002, 2004; Perez et al., 2002). This negative effect not only has been reported for homozygous positive (nn) pigs, but also for Nn individuals compared with non carrier (NN) pigs (Murray and Johnson, 1998).

Therefore, the aim of this study was to analyse the influence of commercial transport conditions on the stress serum parameters of pigs transported to slaughter during different seasons.

MATERIAL AND METHODS

Animals and transport procedures

This field study was carried out following the guidelines of the Council Directive 86/609/EEC, concerning the protection of animals used for scientific research, as well as the European law on animal transportation (Council Regulation 1/2005/EC).

One hundred and sixty-two crossbred pigs (82 females and 80 males), with a live weight of about 98 kg, were randomly chosen among 1 392 pigs transported in seven commercial journeys (five in summer, n = 106 pigs; two in winter, n = 56 pigs). Pigs came from different single-site units (all-in, all-out management system), but were subjected to similar rearing conditions throughout the growing-fattening period, including a 12 h food deprivation before the journey. Pigs were transported to the slaughterhouse by specialized companies according to similar handling and transport practices, using authorized triple-decker vehicles with natural ventilation, drinking nipples and showers, and with average loading density being slightly more reduced in summer than in winter (210 kg/m^2 and 220 kg/m^2, respectively). Each journey was performed transporting pigs from one farm to the slaughterhouse, not mixing animals from different farms, but mixing groups from the same farm both during transport and lairage. Distances were either short (about 70 km) or long (about 900 km), with average durations of 1 h (n = 73 pigs) and 13 h 15 min (n = 89 pigs), respectively. After unloading, pigs were kept in lairage pens, being immediately showered and having free access to drinking water. Lairage time was about 6 h, being the same for short and long transportations, after which pigs were killed according to normal commercial practices.

Samples collection and measurements

For each of the studied pigs one blood sample (baseline sample) was collected via the jugular vein 6 h prior to loading. Sampled pigs were identified for subsequent blood sampling, and were randomly loaded to any of the lorry pens. A second sample was taken immediately after unloading, which was obtained from only 64 pigs since the slaughterhouse wanted to minimize any additional stress that could affect meat quality. A third blood sample was taken at exsanguination. Blood was collected in 10 ml evacuated tubes (BD Vacutainer Systems), was allowed to clot at room temperature, and was centrifuged for 10 min at 75 g. Serum was frozen at –20°C until analysis.

A hair root sample was also obtained from all the studied pigs for the determination of their genotype for the *RYR1* gene (halothane gene). PCR amplification and digestion with restriction enzymes were performed as described by Fujii et al. (1991; HAL-1843, Innovations Foundation). Unfortunately, in 11 cases it was not possible to extract and amplify the DNA of the hair root.

Temperature and relative humidity at loading and unloading were recorded for each transportation. A commercial RIA kit was used for the determination of the serum concentration of cortisol (Diasorin

Inc.) using a 1470 Wizard Automatic Gamma Counter (PerkinElmer Life and Analytical Sciences, Inc.). The detection limit was 0.21 µg/dl according to the manufacturer, and intra and inter assay CV were 5.3% and 7.8%, respectively. Glucose, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), albumin and total protein serum concentrations were measured using spectrophotometric procedures in commercially available kits with a Roche/Hitachi Modular D-P automated chemistry analyser (Roche Diagnostics Division).

Statistical analysis

The data (cortisol, glucose, CPK, LDH, albumin and total proteins serum concentration) at loading, unloading and exsanguination were analysed. Additionally, with the aim to minimize any individual effect on the variables, overall (exsanguination – loading), unloading (unloading – loading) and lairage (exsanguination – loading) differences were also calculated and analysed. Normality was tested for all variables. A mixed model was used, including sex (female vs. male), duration of transport (1 h vs. 13 h 15 min) and season (summer vs. winter) as the main fixed factors, as well as their two-way interactions. Lorry trip (n = 7) was also included in the model as a random effect to take into account the relationship between individual measurements from the same transport. In the analysis of the overall, unloading, and lairage variations, the initial value of the interval was included as a covariable for each animal. The same model was used to study the effect of the halothane gene (homozygous negative [NN] vs. heterozygous [Nn]), and its interactions with the aforementioned main fixed factors. Data from nn individuals were removed from the analysis since their frequency was only 4.6%.

The MIXED procedure of SAS software (SAS Inst.) was used, and the effect of the main factors and two-way interactions were studied (F-test, $P < 0.05$). As the number of comparisons was reduced, probability values were not adjusted. Least-square means (lsmeans) were computed for all main and two-way interactive effects, and separated statistically using pairwise t-tests when a significant ($P < 0.05$) F-test was observed.

RESULTS

Mean temperatures and relative humidities during all journeys were 26.1°C and 39% in summer, and 9.3°C and 61% in winter.

Since our model was unbalanced, lsmeans are provided in all tables to avoid biassed values. With respect to the initial values, males showed higher baseline cortisol levels (Table 1), while albumin and protein levels were higher in the case of females. In addition cortisol ($P < 0.001$) and protein ($P < 0.01$) levels were higher in summer, while glucose and CPK showed higher levels in winter ($P < 0.001$).

Overall mean values at loading, unloading and exsanguination are shown in Table 2. Levels increased during the journey and decreased during the lairage for all variables ($P < 0.001$), with cortisol levels being 3.47 ± 0.19 µg/dl at loading, 8.52 ± 0.28 µg/dl

Table 1. Influence of sex and season on the studied serum variables of pigs before transport (lsmeans ± standard error)

	Sex			Season		
	female (82)[1]	male (80)[1]	P-value	winter (56)[1]	summer (106)[1]	P-value
Cortisol (µg/dl)	3.00 ± 0.21	3.68 ± 0.22	*	2.83 ± 0.24	3.85 ± 0.19	***
Glucose (g/l)	0.56 ± 0.02	0.57 ± 0.02	ns	0.62 ± 0.03	0.51 ± 0.02	***
CPK (IU/l)	2 823 ± 243	2 342 ± 254	ns	3 241 ± 291	1 924 ± 214	***
LDH (IU/l)	1 203 ± 38.34	1 114 ± 40.20	ns	1 201 ± 45.94	1 109 ± 33.75	ns
Albumin (g/l)	43.19 ± 0.49	40.17 ± 0.51	***	41.82 ± 0.58	41.55 ± 0.43	ns
Total proteins (g/l)	73.87 ± 0.60	71.66 ± 0.63	**	71.51 ± 0.72	74.03 ± 0.53	**

CPK = creatine phosphokinase

LDH = lactate dehydrogenase

[1]sample size is provided between brackets

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns: non significant

Table 2. Overall effect of journey and lairage on serum variables of pigs transported to slaughter (lsmeans ± standard error)

	Loading (162)[1]	Unloading (64)[1]	Exsanguination (162)[1]	*P*-value
Cortisol (μg/dl)	3.47 ± 0.19[c]	8.52 ± 0.28[a]	6.96 ± 0.18[b]	***
Glucose (g/l)	0.54 ± 0.03[b]	0.44 ± 0.04[b]	0.86 ± 0.03[a]	***
CPK (IU/l)	2 337 ± 377[c]	7 698 ± 535[a]	6 058 ± 379[b]	***
LDH (IU/l)	1 145 ± 71.58[c]	1 943 ± 102.09[a]	1 549 ± 71.58[b]	***
Albumin (g/l)	41.83 ± 0.51[c]	46.19 ± 0.74[a]	43.48 ± 0.52[b]	***
Total proteins (g/l)	73.23 ± 0.80[c]	79.79 ± 0.50[a]	75.52 ± 0.77[b]	***

CPK = creatine phosphokinase; LDH = lactate dehydrogenase

[1]sample size is provided between bracket

[a,b,c]within a row, means without a common superscript differ significantly

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns: non significant

at unloading, and 6.96 ± 0.18 μg/dl at exsanguination. On the other hand, serum glucose remained constant during the journey (0.54 ± 0.03 g/l at loading, and 0.44 ± 0.04 g/l at unloading) but increased during the lairage (0.86 ± 0.03 g/l; $P < 0.001$).

The increase in cortisol observed between loading and exsanguination (Table 3) was higher in short journeys (4.71 ± 0.31 vs. 3.59 ± 0.29 μg/dl; $P < 0.01$). This increase was already noticeable at unloading, and cortisol levels during lairage remained practically constant in those pigs transported for 1 h, while they decreased in those transported for 13 h 15 min ($P < 0.01$). The increase in serum glucose between loading and slaughter was higher in short transportations (0.54 ± 0.06 vs. 0.17 ± 0.05 g/l; $P < 0.001$). Glycaemia remained almost constant in short journeys, but decreased in long ones. Subsequently, serum glucose during lairage increased in all cases, although more noticeably in pigs transported in the short journeys. CPK and LDH increased during the journey and decreased during lairage, but were not affected by transport duration. Total proteins and albumin levels increased at slaughter with respect to loading, especially in long transportations ($P < 0.001$), and mainly occurring during lairage (0.51 ± 1.46 vs 6.87 ± 1.29 g/l for total proteins).

The influence of season is shown in Table 4. Cortisol levels increased between loading and slaughter mainly in winter transportations (4.79 ± 0.34 vs. 3.52 ± 0.27 μg/dl; $P < 0.01$), subsequently decreasing during lairage. Serum glucose showed no significant differences in the variation between loading and exsanguination, although it decreased between unloading and loading in summer journeys ($P < 0.001$). CPK ($P < 0.001$) and LDH ($P < 0.01$) levels between loading and slaughter increased more markedly in winter (4 545 ± 401 vs. 2 587 ± 324 IU/l for CPK levels), since lairage levels decreased especially in summer. Although albumin and total proteins showed no seasonal differences between loading and exsanguination, albumin increased between loading and unloading, particularly in summer (0.55 ± 0.31 vs. 1.74 ± .033 g/l; $P < 0.01$).

Table 5 shows the significant effects of the interaction between season and duration, as well as season and gender, on the loading-slaughter serum variations. LDH levels increased in all cases, especially in short transportations performed in winter, but with less intensity in males transported in summer ($P < 0.05$). Furthermore, albumin and total proteins also increased in all cases, but particularly in long transportations performed in winter.

Significant interactions during the journey (unloading – loading) and lairage (exsanguination – unloading) are shown in Table 6. Journeys caused an increase in cortisol levels, with the lowest intensity corresponding to males transported in summer. Glucose always decreased, except for short winter journeys. During lairage, CPK ($P < 0.01$) and LDH ($P < 0.05$) recovery was more accentuated in summer, particularly in short journeys and in males. Albumin and total proteins increased during lairage, especially in animals transported long distances in winter.

The halothane gene (Table 7) only affected glucose levels before transport ($P < 0.05$). Significant interactions between halothane gene and duration, and halothane gene and season on the exsanguination – loading variation are shown in Table 8. The response of pigs to journey duration and season was more pronounced in the case of Nn than in NN individuals.

Table 3. Influence of the journey duration on the individual variation of serum parameters of pigs transported to slaughter (lsmeans ± standard error)[1]

	Exsanguination – Loading			Exsanguination – Unloading			Unloading – Loading		
	1 h (73)[2]	> 13 h (89)[2]	*P*	1 h (32)[2]	> 13 h (32)[2]	*P*	1 h (32)[2]	> 13 h (32)[2]	*P*
Cortisol (μg/dl)	4.71 ± 0.31 [135.7]	3.59 ± 0.29 [103.5]	**	0.07 ± 0.51 [0.8]	–1.78 ± 0.47 [–20.89]	**	6.14 ± 0.74 [188.3]	3.99 ± 0.64 [116.7]	*
Glucose (g/l)	0.54 ± 0.06 [90.0]	0.17 ± 0.05 [32.7]	***	0.66 ± 0.09 [103.1]	0.10 ± 0.08 [32.3]	***	0.04 ± 0.06 [6.7]	–0.21 ± 0.05 [–40.4]	**
CPK (IU/l)	3 450 ± 373 [123.3]	3 682 ± 349 [155.6]	ns	–2 042 ± 677 [–24.9]	–1 005 ± 601 [–14.1]	ns	5 414 ± 3 634 [193.4]	4 742 ± 3 104 [200.4]	ns
LDH (IU/l)	361 ± 58.64 [31.1]	305 ± 55.10 [26.3]	ns	–437 ± 105.88 [–18.4]	–406 ± 93.92 [–23.0]	ns	1 214 ± 742 [104.7]	609 ± 634 [52.6]	ns
Albumin (g/l)	2.60 ± 0.38 [6.4]	5.70 ± 0.36 [13.4]	***	1.02 ± 0.95 [1.4]	3.78 ± 0.84 [8.6]	*	1.06 ± 0.34 [2.6]	1.23 ± 0.29 [2.9]	ns
Total proteins (g/l)	2.62 ± 0.55 [3.6]	9.25 ± 0.51 [12.7]	***	0.51 ± 1.46 [1.1]	6.87 ± 1.29 [9.2]	**	1.63 ± 0.76 [2.2]	1.78 ± 0.65 [2.4]	ns

CPK = creatine phosphokinase

LDH = lactate dehydrogenase

[1]for each pig, individual variation was calculated as the final value minus the initial value of the interval

[2](sample size) [variation percentage with respect to the initial value]

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns = non sgnificant

Table 4. Influence of season on the individual variation of serum parameters of pigs transported to slaughter (lsmeans ± standard error)[1]

	Exsanguination – Loading			Exsanguination – Unloading			Unloading – Loading		
	Winter (56)[2]	Summer (106)[2]	*P*	Winter (32)[2]	Summer (32)[2]	*P*	Winter (32)[2]	Summer (32)[2]	*P*
Cortisol (μg/dl)	4.79 ± 0.34 [138.0]	3.52 ± 0.27 [101.4]	**	–0.66 ± 0.42 [–7.8]	–1.05 ± 0.56 [-12,3]	ns	5.28 ± 0.67 [186.6]	4.85 ± 0.71 [126.0]	ns
Glucose (g/l)	0.44 ± 0.09 [71.0]	0.27 ± 0.07 [52.9]	ns	0.48 ± 0.09 [68.6]	0.28 ± 0.10 [107.7]	ns	0.08 ± 0.06 [12.9]	–0.25 ± 0.06 [–49.0]	***
CPK (IU/l)	4 545 ± 401 [140.2]	2 587 ± 324 [134.5]	***	–141 ± 559 [–1.3]	–2 906 ± 730 [–63.2]	**	7 485 ± 3 297 [230.9]	2 671 ± 3 472 [138.8]	ns
LDH (IU/l)	442 ± 62.65 [36.8]	224 ± 50.73 [20.1]	**	–275 ± 86.72 [–10.5]	–568 ± 112.94 [–37.6]	*	1 423 ± 672.87 [118.5]	400 ± 708.55 [36.1]	ns
Albumin (g/l)	4.41 ± 0.40 [10.5]	3.89 ± 0.33 [9.4]	ns	3.31 ± 0.77 [7.8]	1.49 ± 1.01 [3.4]	ns	0.55 ± 0.31 [1.3]	1.74 ± 0.33 [4.2]	**
Total proteins (g/l)	5.53 ± 0.60 [7.7]	6.33 ± 0.48 [8.6]	ns	5.22 ± 1.21 [7.2]	2.15 ± 1.54 [2.8]	ns	0.85 ± 0.69 [1.2]	2.56 ± 0.73 [3.5]	ns

CPK = creatine phosphokinase

LDH = lactate dehydrogenase

[1]for each pig, individual variation was calculated as the final value minus the initial value of the interval

[2](sample size) [variation percentage with respect to the initial value]

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns = non sgnificant

Table 5. Significant effects of the interaction between season and journey duration, and between season and sex, on the individual exsanguination – loading variation of serum parameters of transported pigs (lsmeans ± standard error)[1]

	Winter		Summer		*P*-value
	1 h (30)[2]	> 13 h (26)[2]	1 h (43)[2]	> 13 h (63)[2]	
LDH (IU/l)[3]	546 ± 90.48[a]	338 ± 87.17[ab]	175 ± 75.82[b]	272 ± 67.73[b]	*
Albumin (g/l)	2.12 ± 0.58[c]	6.70 ± 0.57[a]	3.08 ± 0.49[c]	4.70 ± 0.44[b]	**
Total proteins (g/l)	0.14 ± 0.94[d]	10.92 ± 0.82[a]	5.09 ± 0.74[c]	7.57 ± 0.64[b]	***
	Female (29)	Male (27)	Female (53)	Male (53)	
LDH (IU/l)	400 ± 81.06[a]	484 ± 97.75[a]	351 ± 73.16[a]	96.35 ± 70.01[b]	*

LDH = lactate dehydrogenase

[1]for each pig, individual variation was calculated as the final value minus the initial value of the interval

[2]sample size is provided between brackets

[a,b,c,d]within a row, means without a common superscript differ significantly

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 6. Significant effect of the interaction between season and journey duration, and season and sex, on the individual unloading – loading variation, and between season and journey durarion, and season and sex, on the individual exsanguination – unloading variation of serum parameters of transported pigs (lsmeans ± standard error)[1]

	Winter		Summer		*P*-value
	1 h (16)[2]	> 13 h (16)[2]	1 h (16)[2]	> 13 h (16)[2]	
Unloading – Loading					
Glucose (g/l)	0.38 ± 0.10[a]	–0.22 ± 0.07[b]	–0.31 ± 0.09[b]	–0.20 ± 0.08[b]	***
	Female (16)	Male (16)	Female (16)	Male (16)	
Cortisol (μg/dl)	5.66 ± 0.90[a]	4.91 ± 0.98[ab]	7.18 ± 1.04[a]	2.52 ± 0.96[b]	*
Exsanguination – Unloading					
CPK (IU/l)	670 ± 925[a]	–952 ± 633[a]	–4 754 ± 1,017[b]	–1 058 ± 1 034[a]	**
LDH (IU/l)	–76.13 ± 144.62[a]	–473 ± 99.05[bc]	–798 ± 157.45[c]	–339 ± 160.97[ab]	*
Albumin (g/l)	0.75 ± 1.27[b]	5.88 ± 0.89[a]	1.30 ± 1.41[b]	1.69 ± 1.44[b]	*
Total proteins (g/l)	–1.85 ± 2.08[b]	12.30 ± 1.37[a]	2.87 ± 2.21[b]	1.43 ± 2.26[b]	***
	Female (16)	Male (16)	Female (16)	Male (16)	
CPK (IU/l)	–1 057 ± 734[a]	775 ± 832[a]	–1 321 ± 1 089[a]	–4 491 ± 964[b]	**
LDH (IU/l)	–295 ± 115[a]	–254 ± 130[a]	–329 ± 169[a]	–808 ± 149[b]	*

CPK = creatine phosphokinase; LDH = lactate dehydrogenase

[1]for each pig, individual variation was calculated as the final value minus the initial value of the interval

[2]sample size is provided between brackets

[a,b,c]within a row, means without a common superscript differ significantly

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

DISCUSSION

Gender and seasonal effects were observed on the baseline levels, although values (Table 1) may generally be considered as normal for this species and physiological state (Hicks et al., 1998), with glucose levels being lower than those reported by Warriss et al. (1998) possibly due to fasting previous to transport. Furthermore, these authors affirmed that CPK values up to 1 100 IU/l could be considered as normal, although they admitted that levels may be much higher.

Table 7. Influence of the genotype for the halothane gene on serum variables of pigs before transport (lsmeans ± standard error)

	NN (44)[1]	Nn (100)[1]	*P*-value
Cortisol (μg/dl)	3.11 ± 0.26	3.07 ± 0.23	ns
Glucose (g/l)	0.42 ± 0.04	0.58 ± 0.03	*
CPK (IU/l)	2 132 ± 342	2 393 ± 282	ns
LDH (IU/l)	1 146 ± 60.85	1 180 ± 48.21	ns
Albumin (g/l)	40.97 ± 0.89	41.32 ± 0.57	ns
Total proteins (g/l)	74.47 ± 0.73	73.04 ± 0.69	ns

CPK = creatine phosphokinase
LDH = lactate dehydrogenase
[1]sample size is provided between brackets
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns = non sgnificant

Table 8. Significant effects of the interactions between the genotype for the halothane gene and journey duration, and between the genotype for the halothane gene and season, on the individual exsanguination – loading variation of serum parameters of transported pigs (lsmeans ± standard error)[1]

	NN		Nn		*P*-value
	1 h (22)[2]	> 13 h (22)[2]	1 h (44)[2]	> 13 h (56)[2]	
Cortisol (μg/dl)	5.21 ± 0.89[a]	3.67 ± 0.64[b]	5.24 ± 0.43[a]	3.44 ± 0.39[b]	**
Glucose (g/l)	0.11 ± 0.16[ab]	0.08 ± 0.12[b]	0.43 ± 0.08[a]	0.08 ± 0.07[b]	**
Albumin (g/l)	5.21 ± 1.31[ab]	6.14 ± 0.94[a]	2.63 ± 0.63[b]	5.41 ± 0.56[a]	**
Total proteins (g/l)	6.32 ± 1.92[bc]	10.20 ± 1.39[a]	2.42 ± 0.92[c]	8.76 ± 0.82[ab]	***
	Winter[2] (9)	Summer[2] (35)	Winter[2] (35)	Summer[2] (65)	
Cortisol (μg/dl)	4.36 ± 1.10[ab]	4.52 ± 0.55[ab]	5.30 ± 0.36[a]	3.38 ± 0.46[b]	**
Glucose (g/l)	0.33 ± 0.20[a]	–0.14 ± 0.10[c]	0.36 ± 0.06[a]	0.14 ± 0.08[b]	**
LDH (IU/l)	–74.71 ± 236.51[b]	198 ± 117.86[b]	539 ± 76.93[a]	224 ± 98.36[b]	*

LDH = lactate dehydrogenase
[1]for each pig, individual variation was calculated as the final value minus the initial value of the interval
[2]sample size is provided between brackets
[a,b,c]within a row, means without a common superscript differ significantly
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

It is known that transport modifies animals physiology (Broom, 2000), and that lairage helps to recover from this previous stress (Brown et al., 1999a). Serum variables generally followed this expected variation (Table 2), with the highest values being found immediately after transport, and decreasing at the end of lairage.

Variations in serum parameters (Table 3 and 4) were similar to those reported in other studies carried out with similar pigs (Brown et al., 1999a; Perez et al., 2002). Variations of cortisol, despite possible diurnal variations, and glucose (Table 3) suggest that short journeys did not permit a total recovery from the initial stress caused by loading, which is known to have a negative impact on the welfare of animals (Broom, 2000) independently from the loading method used (Brown et al., 2005). Furthermore, Bradshaw et al. (1996) found a raise in cortisol levels 30 min after loading, and a gradual decrease after 4.5 h of transportation, and Apple et al. (2005) found that cortisol levels matched up with an increase in plasmatic glucose. These assertions would justify our results, so that, in the case of short transportations, initial stress caused by

loading was aggravated by unloading and mixing of pigs during lairage. Additionally, initial cortisol and glucose values could not be recovered during lairage, what would explain the dramatic increase in glucose levels at exsanguination with respect to those at loading and unloading observed in short journeys (Table 2). The increase of glycaemia has been attributed to an energy demand caused by the handling and the movement of the animals (Becker et al., 1989), although the increase in cortisol and glucose levels were only found in those pigs with a submissive behaviour (Hicks et al., 1998).

The evolution of albumin and total proteins (Table 3) might indicate some dehydration although, surprisingly, it became more apparent during the lairage of pigs transported the longest distances. It has been described that pigs drink little water during the journey (Lambooy et al., 1985), and that actual water usage during lairage might be related to pigs playing during the initial exploration period (Brown et al., 1999b). In our case pigs remained 6 h in lairage on average, time sufficient for a total recovery from transport stress (Warriss et al., 1992). Taking into consideration the findings of these authors, and although no behavioural observations were made in the present study, it might have been possible that water consumption during transport was reduced, what would account for the increase in albumin and total protein levels. Moreover, our results would indicate a recovery from the physical stress caused by the journey, thus decreasing CPK and LDH levels during lairage. Brown et al. (1999a) did not find any differences in albumin and protein levels caused by transport, but described a similar CPK activity.

Season had an effect on the stress during transportation (Table 4). As an overall estimation, it might be affirmed that stress from loading to slaughter was lower in summer, agreeing with other Spanish studies related to mortality, carcass characteristics (Gosalvez et al., 2006), and meat quality (Guardia et al., 2005), but disagreeing with Vecerek et al. (2006), what might be due to climatic differences between the regions where studies were carried out. Cortisol variation might indicate that winter transportations were slightly more stressful, with lairage recovery being poorer than in summer. Other studies performed in Spanish conditions also found higher cortisol levels in winter (Gispert et al., 2000), which was attributed to the fact that Spanish livestock hauliers adapt to summer conditions, increasing night transportations, reducing loading densities or showering pigs. Our results agree with these authors, as well as with those of Baldwin and Stephens (1973), who found higher cortisol levels in cold climatic conditions related to the energy demand to maintain body temperature. Furthermore, winter increase in cortisol levels was accompanied by a higher muscular effort, agreeing with Gispert et al. (2000) although, in our case, the strong individual variability in CPK and LDH made differences not significant. Nonetheless, muscular recovery during lairage in our study was more reduced in those pigs transported in winter, suggesting that the detrimental effect of transport, in this season, would extend to the lairage period. The higher increase in albumin levels found in summer transportations would indicate a certain degree of dehydration during this season.

An additive effect between season and the rest of factors was observed in the loading-slaughter serum variations (Table 5) highlighting, in winter conditions, the negative impact of short transports on the muscular activity of pigs. Independently from season, the higher stress reactivity in the case of females would agree with other authors (Van der Wal et al., 1999; Perez et al., 2002). A seasonal effect on the dehydration levels was observed, with long winter journeys being the most detrimental for pigs and mainly appearing during lairage (Table 6). The evolution of cortisol and glucose levels during the journey, and the evolution of CPK and LDH during lairage, would confirm the effect of transport on females stress reactivity.

No differences between the two studied genotypes existed before loading (Table 7), except for the serum glucose levels observed in Nn pigs. Table 8 suggests an additive effect between genetics and journey duration, and between genetics and season, reflecting differences in the stress response of Nn individuals with respect to NN individuals, the latter having a more uniform response. These results would agree with those of Fabrega et al. (2002) but would disagree with Gispert et al. (2000). On the other hand, variations in albumin and total protein levels would suggest that NN pigs experienced higher dehydration levels.

CONCLUSIONS

Transports generally increased the stress of slaughter pigs, subsequently decreasing during lairage. Nevertheless, the effect was largely modified by the studied factors. Short and winter transports were more stressful, with lairage recovery being poorer than in long and summer ones. Transports

also caused some dehydration, increasing during lairage especially in those pigs transported for long distances. A higher stress reactivity was detected in females. Genetics modulated the influence of the rest of factors, with Nn individuals having a rougher reaction to short and winter transports, although they became less dehydrated. Therefore, it might concluded that an adaptation of slaughter transports to seasonal differences appears essential in order to minimize the stress of transport and its negative consequences on the welfare of slaughter pigs. Moreover, an improvement in stress resistance could be obtained by controlling the halothane gene of pigs. Nevertheless, new studies would be necessary.

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REFERENCES

Abbott T.A., Guise H.J., Hunter E.J., Penny R.H.C., Bayne P.J., Easby C. (1995): Factors influencing pig deaths during transit: an analysis of drivers' reports. Animal Welfare, 4, 29–40.

Apple J.K., Kegley E.B., Maxwell C.V. Jr., Rakes L.K., Galloway D., Wistuba T.J. (2005): Effects of dietary magnesium and short-duration transportation on stress response, *post mortem* muscle metabolism and meat quality of finishing swine. Journal of Animal Science, 83, 1633–1645.

Baldwin B.A., Stephens D.B. (1973): The effects of conditioned behaviour and environmental factors on plasma corticosteroid levels in pigs. Physiology and Behaviour, 10, 267–274.

Becker B.A., Mayes H.F., Hahn G.L., Nienaber J.A., Jesse G.W., Anderson M.E., Heymann H., Hedrick H.B. (1989): Effect of fasting and transportation on various physiological parameters and meat quality of slaughter hogs. Journal of Animal Science, 67, 334–341.

Bradshaw R.H., Parrott R.F., Goode J.A., Lloyd D.M., Rodway R.G., Broom D.M. (1996): Behavioural and hormonal responses of pigs during transport: effect of mixing and duration of journey. Animal Science, 62, 547–554.

Broom D.M. (2000): Welfare assessment and welfare problem areas during handling and transport. In: Grandin T. (ed.): Livestock Handling and Transport. 2nd ed. CAB International, Wallingford, U.K. 43–61.

Brown S.N., Knowles T.G., Edwards J.E., Warriss P.D. (1999a): Behavioural and physiological responses of pigs to being transported for up to 24 hours followed by six hours recovery in lairage. The Veterinary Record, 145, 421–426.

Brown S.N., Knowles T.G., Edwards J.E., Warriss P.D. (1999b): Relationship between food deprivation before transport and aggression in pigs held in lairage before slaughter. The Veterinary Record, 145, 630–634.

Brown S.N., Knowles T.G., Wilkins L.J., Chadd S.A., Warriss P.D. (2005): The response of pigs to being loaded or unloaded onto commercial animal transporters using three systems. The Veterinary Journal, 170, 91–100.

Council Directive 1986/609/EEC (1986): On the approximation of laws, regulations, and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Official Journal of the European Union L 358, 18/12/1986, pp. 0001–0028.

Council Regulation (EC) No 1/2005 (2005): 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 3, 22/12/2004, pp. 0001–0044.

dalla Costa O.A., Faucitano L., Coldebella A., Ludke J. V., Peloso J.V., dalla Roza D., Paranhos da Costa M.J.R. (2006): Effects of season of the year, truck type and location on truck on skin bruises and meat quality in pigs. Livestock Science, 107, 29–36.

Fabrega E., Manteca X., Font J., Gispert M., Carrion D., Velarde A., Ruiz de la Torre J.L., Diestre A. (2002): Effects of halothane gene and pre-slaughter treatment on meat quality and welfare from two pig crosses. Meat Science, 62, 463–472.

Fabrega E., Manteca X., Font J., Gispert M., Carrion D., Velarde A., Ruiz de la Torre J.L., Diestre A. (2004): A comparison of halothane homozygous negative and positive pietrain sire lines in relation to carcass and meat quality, and welfare traits. Meat Science, 66, 777–787.

Fujii J., Otsu K., Zorzato F., De Leon S., Khanna V.K., Weilter J.E., O'Brien P.J., MacLennan D.H. (1991): Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253, 448–451.

Geers R., Bleus E., Van Schie T., Ville H., Gerard H., Janssens S., Nackaerts G., Decuypere E., Jourquin J. (1994): Transport of pigs different with respect to halothane gene: stress assessment. Journal of Animal Science, 72, 2552–2558.

Gispert M., Faucitano L., Oliver M.A., Guardia M.D., Coll C., Siggens K., Harvey K., Diestre A. (2000): A survey of pre-slaughter conditions, halothane gene frequency, and carcass and meat quality in five Spanish pig commercial abattoirs. Meat Science, 55, 97–106.

Gosalvez L.F., Averos X., Valdelvira J.J., Herranz A. (2006): Influence of season, distance and mixed loads on the physical and carcass integrity of pigs transported to slaughter. Meat Science, 73, 553–558.

Grandin T. (2000): Introduction: Management and Economic Factors of Handling and Transport. In: Grandin T. (ed.): Livestock Handling and Transport. 2nd ed. CAB International, Wallingford, U.K. 1–14.

Guardia M.D., Estany J., Balasch S., Oliver M.A., Gispert M., Diestre A. (2004): Risk assessment of PSE condition due to pre-slaughter conditions and *RYR1* gene in pigs. Meat Science, 67, 471–478.

Guardia M.D., Estany J., Balasch S., Oliver M.A., Gispert M., Diestre A. (2005): Risk assessment of DFD meat due to pre-slaughter conditions in pigs. Meat Science, 70, 709–716.

Hicks T.A., McGlone J.J., Whisnant C.S., Kattesh H.G., Norman R.L. (1998): Behavioral, endocrine, immune and performance measures for pigs exposed to acute stress. Journal of Animal Science, 76, 474–483.

Lambooy E., Garssen G.J., Walston P., Mateman G., Merkus G.S.M. (1985): Transport of pigs by car for 2 days: some aspects of watering and load density. Livestock Production Science, 13, 289–299.

Mota-Rojas D., Becerril M., Lemus C., Sanchez P., Gonzalez M., Olmos S.A., Ramirez R., Alonso-Spilsbury M. (2006): Effects of mid-summer transport duration on pre- and post-slaughter performance and pork quality in Mexico. Meat Science, 73, 404–412.

Murray A.C., Johnson C.P. (1998): Impact of halothane gene on muscle quality and pre-slaughter deaths in Western Canadian pigs. Canadian Journal of Animal Science, 78, 543–548.

O'Neill D.J., Lynch P.B., Troy D.J., Buckley D.J., Kerry J.P. (2003): Influence of the time of the year on the incidence of PSE and DFD in Irish pigmeat. Meat Science, 64, 105–111.

Palacio J., Garcia-Belenguer S., Gascon F.M., Liste F., Ortega C., Lobera B., Martin-Maestro I., Angel J.A., Lles J.C., Bayo F. (1996): Mortalidad durante el transporte a un matadero en ganado porcino. Investigacion Agraria: Produccion y Sanidad Animal, 11, 159–169.

Perez M.P., Palacio J., Santolaria M.P., Acena M.C., Chacon G., Gascon M., Calvo J.H., Zaragoza P., Beltran S., Garcia-Belenguer S. (2002): Effect of transport time on welfare and meat quality in pigs. Meat Science, 61, 425–433.

Rundgren M., Lundstrom K., Edfors-Lilja I., Juneja R.K. (1990): A within-litter comparison of the three halothane genotypes. 1. Piglet performance and effects of transportation and amperozide treatment at 12 weeks of age. Livestock Production Science, 26, 137–153.

Smith L.P., Allen W.M. (1976): A study of weather conditions related to the death of pigs during and after their transportation in England. Agricultural Meteorology, 16, 115–124.

Van der Wal P.G., Engel B., Reimert H.G.M. (1999): The effect of stress, applied immediately before stunning, on pork quality. Meat Science, 53, 101–106.

Vecerek V., Malena M., Malena M. Jr., Voslarova E., Chloupek P. (2006): The impact of the transport distance and season on losses of fattened pigs during transport to the slaughterhouse in the Czech Republic in the period from 1997 to 2004. Veterinarni Medicina, 51, 21–28.

Warriss P.D. (1998): The welfare of slaughter pigs during transport. Animal Welfare, 7, 365–381.

Warriss P.D., Brown S.N., Edwards J.E., Anil M.H., Fordham D.P. (1992): Time in lairage needed by pigs to recover from the stress of transport. The Veterinary Record, 131, 194–196.

Warriss P.D., Brown S.N., Knowles T.G., Edwards J.E., Kettlewell P.J., Guise H.J. (1998): The effect of stocking density in transit on the carcass quality and welfare of slaughter pigs: 2. Results from the analysis of blood and meat samples. Meat Science, 50, 447–456.

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