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Influence of lairage time on some welfare and meat quality parameters in pigs

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Abstract – A total of 150 Large White cross Landrace pigs (110-120 kg) of both sexes were used to investigate the effects of three different lairage times (0 h, 3 h, 9 h). Blood samples were collected at exanguination and cortisol, glucose, lactate, muscle enzymes and haematological parameters were determined. Post-mortem measurements of muscle pH were taken at 20 min, 2 h and 24 h from *Longissimus thoracis* and *Semimembranosus*. Lairage time showed a significant effect on pH_{24}, internal muscle reflectance using the fibre optic probe (FOP_{24}), red blood cells, neutrophils and lymphocytes, glucose and enzymatic activities. Changes in blood profile and meat quality parameters indicated that three hours of lairage in Spanish commercial conditions may reduce the amount of stress exhibited by pigs at slaughter and better meat quality can be obtained. No lairage or an excessively long lairage period without food may compromise animal welfare and meat quality.

lairage / welfare / stress / meat quality / pigs

Résumé – Influence du temps d'attente avant l'abattage sur le bien-être et la qualité de la viande du porc. Cent cinquante porcs de race Large White × Landrace (110–120 kg) ont été utilisés dans le but d'étudier l'effet de trois temps d'attente différents avant l'abattage (0 h, 3 h, 9 h).

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Des prélèvements de sang ont été réalisés au moment de la saignée pour l'analyse de la concentration de cortisol, glucose, lactate, l'activité des enzymes d'origine musculaire et différents paramètres hématologiques. Le pH des muscles *Longissimus thoracis* et *Semimembranosus* a été mesuré à 20 min, 2 h et 24 h après la mort des animaux. Le temps d'attente à l'abattage a montré un effet significatif ($p < 0.05$) sur le pH à 24 h (pH_{24}), la reflectance interne du muscle à 24 h (Fibre Optic Probe: FOP_{24}), la numération érythrocytaire, les pourcentages de neutrophiles et lymphocytes, la glycémie et l'activité enzymatique. Tous les changements sanguins et des paramètres de la qualité de la viande signalent que dans les conditions normales du commerce en Espagne, un temps d'attente de trois heures à l'abattoir pourrait réduire le niveau de stress montré pour les porcs à l'abattage et améliorer la qualité de la viande. De même l'abattage des animaux sans attente comme l'abattage après un très long temps à l'abattoir sans nourriture pourrait compromettre fortement le bien-être et la qualité de la viande de porc.

attente à l'abattage / bien-être / stress / qualité de la viande / porc

1. INTRODUCTION

In general, pigs are reared in environments with low levels of stimulation and the animals may have difficulty coping with new environment stimuli. From the farm to slaughter, pigs encounter many different stimuli such as loading and unloading, being transported or being mixed with unfamiliar pigs [2, 21]. All these stimuli may impose stress which impairs welfare.

Animal welfare can be assessed by using indicators of poor welfare, such as behavioural and physiological changes occurring in stressful situations [12]. Plasma cortisol increase, hyperglycemia, leucocytosis with neutrophilia, lymphopaenia and eosinopaenia are evident signs of stress response [6].

Pre-slaughter handling also affects muscle glycogen metabolism and may increase the incidence of DFD (dry, firm, dark) or PSE (pale, soft, exudative) meat [1, 11]. DFD meat is caused by depletion of muscle glycogen before slaughter, which limits post-mortem muscle acidification. In PSE meat, pre-slaughter stress causes the muscles to acidify at a rapid rate whilst the carcass is still hot. This impairment in meat quality may be measured by a fall in muscle pH post-mortem [17]. The tendency to develop rapid acidification is largely influenced by the genotype of the pig [16] and feed withdrawal [7, 9].

A period of rest in lairage is generally recommended to allow the pigs to recover from transport and associated handling. In spite of this, legislation (EU Directive 93/119/EC) [10] does not suggest any precise time for lairage. With regards to meat quality, a period of rest in lairage between two and four hours is recommended since different studies have shown a decreasing tendency to PSE and an increasing tendency to DFD with longer lairage times [11, 18, 27, 30]. The beneficial effect of lairage time on PSE-related traits seems to be more pronounced in genetically stress susceptible pigs than in stress resistant pigs [7, 26].

From the point of view of animal welfare, the effects of different lairage times are not well defined. Geverink et al. [14] recommended that the animals which are not sedated, be slaughtered immediately upon arrival. Santos et al. [26] recommended that animals be slaughtered as quickly as possible in warm environments and after two or three hours lairage at moderate temperatures. Warris et al. [29], under UK conditions and in pigs of both sexes weighing around 89 kg, also observed that two or three hours of rest reduced cortisol and β-endorphin levels and therefore recommended it with regards to welfare. But in a later study, Warris et al. [30] observed that

the changes in the blood profile indicated that overnight lairage reduced the amount of stress exhibited by pigs, although an important interaction between lairage time and transport time was also detected.

In Spain, most pigs have to travel less than 50 km and usually under warm temperatures. Mixing unfamiliar pigs from different plants is always avoided. However, pigs from different pens but from the same plant are unavoidably mixed during transportation. Feed is withdrawn approximately 12 h before loading and pigs are rarely fed during lairage in the abattoir, since they rarely rest more than 12 h before slaughter.

The purpose of this investigation was to study the effect of different lairage times on pig welfare and subsequent meat quality, under the most frequent environmental and commercial conditions in Spain. A single non replicated experiment has been developed as a pilot study which could highlight a potential area of further, rigorous, replicated studies.

2. MATERIALS AND METHODS

2.1. Experimental design

A total of 150 Large White cross Landrace pigs of both sexes weighing 110–120 kg, were included in this study. All the animals were derived from the same breeding company and the same barn, where 300 pigs were fattened in 12 pens, which hold 25 pigs of both sexes in each. All the animals were transported to the slaughterhouse in two trips, half of them in the morning and the other half in the previous evening. The farm was situated 20 km from the slaughterhouse. Only 100 pigs transported together in the morning were included in the study, 50 were sacrificed immediately on arrival and 50 were sacrificed three hours later. Only 50 pigs transported together the previous evening were included in the study and they stayed in lairage for nine hours. Then, for lairage, animals were held in three batches of 50 animals subjected to zero, three and nine hours of lairage. These times were chosen from the most habitual lairage times used in Spanish commercial conditions in a standard industrial abattoir. Travel conditions and handling were the same for all 150 pigs included in the study (lorry, driver, density: 235 kg/m^2). At the moment of loading, the animals had been fasting for 12 h. The animals were loaded by means of a tailgate lift and unloaded by a ramp (15º slope). The animals were always herded using pig boards and without the use of sticks or goads. Mean live weight of the groups when they arrived at the abattoir were 114.5, 115.2 and 113.5 kg, respectively. The 0 h and 3 h groups arrived at the slaughterhouse in the morning when the production chain was at maximum performance and the 9 h group arrived during the previous evening and waited overnight. The 3 h and 9 h groups were introduced into two identical slaughter pens respectively and lairage density was 0.48 m^2/animal for both batches. Animals were showered during the first 15 min of lairage. They had access to water but not food. Then, total fasting time, including the journey and lairage times was 13, 16 and 22 h, respectively. All these times are according to legislation. The experiment was carried out in the spring with an outdoor temperature of 15 ºC and an indoor temperature of 20 ºC. All the animals were slaughtered within an hour on the same day and in commercial conditions after stunning (500 V, 1.8 A) using a restrainer system. Exanguination was carried out with the animal suspended from its left hind leg. The abattoir had an output of 350 000 pigs a year and a production line speed of 300 pigs per hour.

2.2. Behavioural observations

A video recorder monitored behaviour during the first 3 h of lairage in order to compare the behaviour of the pigs kept overnight

at the plant (9 h group) and those kept during the morning (3 h group). The number of animals resting (sitting and lying down) was recorded punctually every 10 min. Skin damage was assessed as an index of agonistic behaviour before slaughter. A scale of 1 to 5 was used, with 1 indicating no skin blemish and 5 indicating a severe skin blemish [28].

2.3. Blood measurements

Blood samples were collected at exanguination and were kept refrigerated until arrival at the laboratory for immediate processing.

Haematological parameters: packed cell volume, hemoglobine, red blood cells and white blood cells were analysed immediately with an automatic counter (Sysmex F-800, TOA Medical Electronics Co; Ltd., Japan). For differential leukocyte counts, stained smears (Wright's stain) were examined under a light microscope and percentage of lymphocytes, neutrophils and eosinophils were determined.

EDTA plasma and serum were obtained quickly by centrifugation and aliquots were frozen (–30 ºC) for subsequent analysis of cortisol, lactate, glucose and enzymatic activity of creatine kinase (CK), lactic dehydrogenase (LDH), aspartate amino transferase (AST) and alanine amino transferase (ALT). Plasma (EDTA K3) cortisol levels were measured by radioimmunoassay (CT-RIA-I125, Biolink 2000 S.L., Pi i Molist 133, Barcelona, Spain). Plasma (EDTA KF) lactate concentration was measured using a Sigma Diagnostics kit and a spectrophotometer (Lambda 5, PerkinElmer & Co GmbH, 7770 Überlinger, Germany). Serum enzyme activity levels and glucose concentration were analysed by a multianalyser spectrophotometer (Technicon RA-500, Swords Co, Ltd, Dublin, Ireland) using Bayer reagents.

2.4. Meat quality measurements

Post-mortem measurements of muscle pH were taken at 20 min (pH_0), 2 h (pH_2) and 24 h (pH_{24}) from *Longissimus thoracis* (LT) and at 2 h and 24 h from *Semimembranosus* (SM), with a portable pHmeter (Crison-507, 08328 Alella, Spain) and penetration electrode. The internal muscle reflectance, using the fibre optic probe (FOP) [8], was measured in both muscles at 24 h post-mortem. LT and SM measurements were recorded at 3 cm depth, in the region of the last rib and from the right free limb, respectively. These measurements were taken in order to detect PSE and DFD meat [8, 16, 30]. PSE meat is characterised by high light scattering and a rapid acidification ($FOP_{24} > 39$; $pH_{45min} < 5.9$; $pH_2 < 5.7$; $pH_{24} < 5.6$). DFD meat is characterised by low light scattering caused by high ultimate pH values ($FOP_{24} < 19$; $pH_{24} > 6.00$).

Data corresponding to hot carcass weight were also collected.

2.5. Genetic study

Blood from all the animals was analysed in order to detect stress-susceptible pigs. A DNA test for the Porcine Stress Syndrome was performed typing the skeletal ryanodine receptor gene (ryr-1). This method was described by Fugii et al. [13] and modified by Calvo et al. [4]. Genomic DNA was extracted according to a previously described procedure [20].

A 199 bp fragment (including the mutation of the ryr-1 gene: exon 4 contains the 1666 mutation C-T, X68247 Genebank) was amplified with the set of primers. The primers were designed as follows: 5'GTTCCCTGTGTGTGTGC-3' (forward primer) and 5'ATTCACCGGAGTGGAGT-3' (reverse primer). The amplification was carried out in a final volume of 50 µL, containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each of dATP, dTTP, dGTP and dCTP, 4 pmol of

each primer, 20 ng of template DNA, and 1.5 U of Taq polymerase (Promega Corp, Madison, WI, USA). The DNA was amplified in a Biometra Thermal cycler (Biometra Ltd, Kent, UK). Thirty cycles were performed with the following step-cycle profile: strand denaturation at 94 ºC for 1 min, primer annealing at 72 ºC for 30 s. An initial denaturation at 94 ºC for 5 min was performed to improve the final result. Electrophoresis of a 10 µL portion of the amplification was carried out for 45 min at 100 V in 2% agarose gel containing ethidium bromide (1 µg/mL) in TBE buffer. The DNA fragment was visualised by UV transillumination.

In order to obtain the three different genotypes (CC: stress resistant animals, CT: carrier animals, TT: stress susceptible animals) digestion of the PCR fragment was done and one polymorphic recognition site was revealed with the enzyme BsiHKA I. This digestion was carried out at 60 ºC during 2 h 30 min, resulting in a fragment of 199 bp (allele C) or 165 bp or 34 bp (allele T).

2.6. Statistical analyses

Data were analysed using the statistic program SPSS v. 9.0. A multiple analysis of variance (general linear model) was used to examine the effect of lairage time (L), sex (S) and genotype (G) on welfare and meat quality parameters. Interactions between explanatory variables were also included ($L \times S$, $L \times G$; $S \times G$). The scheffe test was applied for multiple comparisons.

The relationship between variables was also studied by using the Pearson correlation with a signification level of $p < 0.05$.

3. RESULTS

No deaths were registered during transport or lairage among the animals in this study.

No significant interactions were detected in the study, so only least square means from lairage, genotype and sex are presented in the tables.

3.1. Effect of lairage time on blood measurements

With respect to haematological parameters (Tab. I), there were significant differences between groups in the percentage of lymphocytes and neutrophils. The 3 h and 9 h lairage groups presented signs of lymphopaenia and neutrophilia. From red

Table I. Least square means (standard error) of the hematological parameters in relation to lairage time, $n = 50$ animals per group.

	Group 0 h	Group 3 h	Group 9 h	Statistical significance
Red blood cells (10^6/µL)	9.0 (0.2)[a]	9.5 (0.3)[a]	9.9 (0.3)[b]	$P < 0.05$
Hemoglobine (g/dL)	14.3 (0.3)	14.9 (0.3)	14.7 (0.4)	NS
Packed cell volume (%)	40.4 (0.9)	41.7 (1.2)	41.3 (1.5)	NS
White blood cells (10^3/µL)	29.4 (1.6)	31.6 (1.9)	28.9 (2.4)	NS
Neutrophiles (%)	46.7 (2.6)[a]	60.0 (3.1)[b]	58.8 (4.1)[b]	$P < 0.05$
Lymphocytes (%)	50.1 (2.5)[a]	36.9 (2.9)[b]	38.5 (3.8)[b]	$P < 0.05$
Eosinophiles (%)	0.3 (0.1)	0.2 (0.2)	0.3 (0.2)	NS

Different letters indicate significant differences between groups.

cell parameters, only the RBC count showed significant differences, the number of cells being higher in the group given 9 h lairage compared to the other two groups.

Table II presents the means of biochemical parameters in relation to lairage time. Glucose concentration was significantly lower in the group subjected to 9 h lairage and the level of cortisol was significantly higher in the group slaughtered on arrival, but no significant correlation was obtained between glucose and cortisol concentrations. Plasma enzymatic activities of ALT, AST, CK and LDH increased as lairage time increased. The four enzymes were significantly correlated between them.

3.2. Effect of lairage time on meat quality measurements

The results of pH and internal muscle reflectance (FOP) in LT and SM muscles depending on waiting times in the abattoir pens are shown in Table III. Significant differences can be noted between the animals slaughtered on arrival and those given nine hours lairage in ultimate pH measurements. The pH-fall was less pronounced in the 9 h group, which presented the highest pH_{24} in both muscles. There was also significant difference between groups in FOP_{24} measurement but only in the LT muscle, showing the highest values in the 0 h group.

A significant negative correlation between pH_{24} and FOP_{24} was found in LT and SM muscles (LT: $r = -0.38$; SM: $r = -0.51$). A significant correlation was also observed between pH_{24} from both muscles ($r = 0.77$). No significant differences were found in carcass weight between the groups.

The percentages of pigs presenting extreme values of ultimate pH or FOP are shown in Table IV. The percentages of pigs presenting $pH_{24} < 5.60$ and $FOP_{24} > 39$ at the same time in any muscle were 24, 14 and 10% for the 0 h, 3 h and 9 h groups, respectively. The percentages of pigs presenting $pH_{24} > 6.00$ and FOP < 19 at the same time in any muscle were 0, 4 and 20% for the 0 h, 3 h and 9 h groups respectively. There was a tendency for the percentage of pigs with PSE characteristics to decrease, whereas the percentage of pigs with DFD characteristics increased, as lairage time increased.

Table II. Least square means (standard error) of the biochemical parameters in relation to lairage time, $n = 50$ animals per group.

	Group 0 h	Group 3 h	Group 9 h	Statistical significance
Cortisol (ng/mL)	98.7 (4.7)[a]	77.8 (5.5) [b]	89.1 (7.2)	$P < 0.05$
Glucose (mg/dL)	78.2[a] (6.2)	64.7 (6.8)	43.6[b] (8.8)	$P < 0.05$
Lactate (mg/dL)	137.7 (9.1)	150.7 (9.7)	156.7 (12.9)	NS
ALT (U/L)	52.4[a] (3.0)	59.7[a] (3.5)	90.5[b] (4.6)	$P < 0.001$
AST (U/L)	103[a] (21)	113[a] (19)	611[b] (32)	$P < 0.001$
CK (U/L)	7692[a] (4455)	14918[b] (3931)	74691[c] (6820)	$P < 0.01$
LDH (U/L)	2052[a] (570)	2894[b] (503)	7490[c] (873)	$P < 0.05$

Different letters indicate significant differences between groups. ALT: alanine amino transferase, AST: aspartate amino transferase, CK: creatine kinase, LDH: lactic dehydrogenase.

Table III. Least square means (standard error) of the meat quality parameters in relation to lairage time, $n = 50$ animals per group.

	Group 0 h	Group 3 h	Group 9 h	Statistical significance
pH_0 LT	6.45 (0.07)	6.39 (0.08)	6.16 (0.11)	NS
pH_2 LT	6.14 (0.08)	6.01 (0.08)	5.97 (0.11)	NS
pH_{24} LT	5.58 (0.06)[a]	5.63 (0.06)	5.89 (0.08)[b]	$P < 0.05$
pH_2 SM	6.06 (0.08)	5.92 (0.08)	5.84 (0.11)	NS
pH_{24} SM	5.54[a] (0.06)	5.59 (0.06)	5.83[b] (0.08)	$P < 0.05$
FOP_{24} LT	35.1[a] (2.96)	25.1[b] (2.96)	19.8[b] (3.58)	$P < 0.05$
FOP_{24} SM	31.9 (3.03)	30.9 (3.03)	28.4 (3.7)	NS
Skin damage	1.18 (0.14)	1.49 (0.14)	1.61 (0.15)	NS
Carcass weight (kg)	91.4 (2.0)	92.3 (1.8)	89.4 (3.1)	NS

Different letters indicate significant differences between groups. LT: *Longissimus thoracis*; SM: *Semimembranosus*; FOP: Fibre Optic Probe.

Table IV. Percentage of animals with signs of PSE (pale, soft, exudative) or DFD (dry, firm, dark) in relation to lairage time.

	PSE				DFD			
	$pH_{24} < 5.60$		$FOP_{24} > 39$		$pH_{24} > 6.00$		$FOP_{24} < 19$	
	LT	SM	LT	SM	LT	SM	LT	SM
Group 0 h	64	72	18	24	0	0	0	0
Group 3 h	60	70	6	14	6	6	4	0
Group 9 h	0	26	10	4	16	20	26	20

LT: *Longissimus thoracis*; SM: *Semimembranosus*; FOP: Fibre Optic Probe.

3.3. Effect of sex and genotype

The genetic study showed 16% dominant homozygotes (CC: 199 bp), 79% heterozygotes (CT: 199 bp + 170 bp + 29 bp) and 5% recessive homozygotes (TT: 170 bp + 29 bp). Distribution by sex was 49% females and 51% males.

Multiple analysis of variance showed a significant effect of genotype on plasma cortisol concentration, plasma ALT, AST, CK and LDH enzyme activities and pH_0 and pH_2 from LT muscle. Table V shows least square means from these parameters but the relationship between lairage time and stress susceptibility could not be demonstrated because there were not enough animals in each group.

Sex (Tab. VI) showed a significant effect on Hb, plasma enzyme activities, pH_2 LT and FOP_{24} SM, with blood parameters higher in females and muscle parameters higher in males.

Table V. Least square means (standard error) of the parameters affected by genotype.

	Stress resistant animals (n = 24)	Carrier animals (n = 118)	Stress susceptible animals (n = 8)	Statistical significance
Cortisol (ng/mL)	75.9[a] (5.3)	88.7 (2.3)	104.1[b] (10.3)	$P < 0.05$
ALT (U/L)	57[a] (3.4)	55[a] (1.5)	98[b] (6.6)	$P < 0.001$
AST (U/L)	166[a] (23.9)	157[a] (10.5)	750[b] (52.0)	$P < 0.001$
CK (U/L)	18970[a] (5041)	20831[a] (2207)	83916[b] (11033)	$P < 0.001$
LDH (U/L)	3449[a] (645)	3300[a] (282)	7415[b] (1413)	$P < 0.05$
pH_0 LT	6.42[a] (0.08)	6.29[b] (0.04)	6.31 (0.15)	$P < 0.05$
pH_2 LT	6.22[a] (0.08)	6.01 (0.04)	5.85[b] (0.16)	$P < 0.05$

Different letters indicate significant differences between groups. LT: *Longissimus thoracis*; ALT: alanine amino transferase; AST: aspartate amino transferase; CK: creatine kinase; LDH: lactic dehydrogenase.

Table VI. Least square means (standard error) of the parameters affected by sex.

	Female (n = 73)	Male (n = 77)	Statistical significance
Hemoglobine (g/dL)	14.97 (0.31)	14.28 (0.29)	$P < 0.05$
ALT (U/L)	80 (3.2)	52 (3.0)	$P < 0.001$
AST (U/L)	414 (21)	161 (20)	$P < 0.001$
CK (U/L)	49420 (4441)	17949 (4379)	$P < 0.001$
LDH (U/L)	5559 (569)	2911 (560)	$P < 0.01$
pH_2 LT	5.92 (0.08)	6.17 (0.07)	$P < 0.05$
FOP_{24} LT	26.22 (3.16)	30.73 (2.40)	$P < 0.05$

Different letters indicate significant differences between groups. LT: *Longissimus thoracis*; ALT: alanine amino transferase; AST: aspartate amino transferase; CK: creatine kinase; LDH: lactic dehydrogenase; FOP: Fibre Optic Probe.

3.4. Behavioural results

The percentage of animals resting increased in proportion to time in lairage and no differences between groups were detected (Fig. 1). Non significant skin damages were detected in pigs at slaughter (Tab. III).

4. DISCUSSION

The impact of lairage time may vary according to the specific situation in each abattoir and in relation to the amount of stress experienced during transportation [7]. That is one of the reasons why it is very important to work in commercial conditions in order to

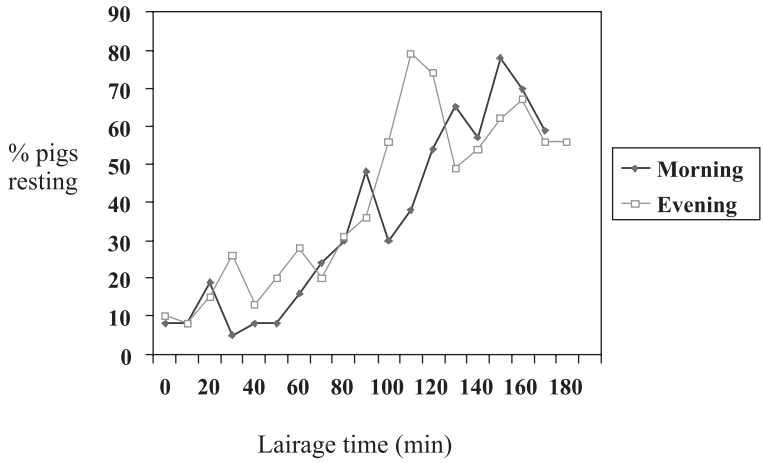

Figure 1. Mean percentage of animals resting (sitting and lying down) during lairage time in the morning (group 3 h) or in the evening (group 9 h).

obtain practical results. This study was carried out using the most frequent conditions found in Spain: short transport at warm temperatures, 12 h fasting before transport and a maximum of 12 h lairage without food. Lairage time can vary from 0 to 12 h, but the most frequent times are: 0 h for pigs that arrive at the abattoir when the slaughter chain is functioning, 2–3 h for pigs that arrive just before the slaughter chain begins functioning and 8–9 h for pigs that arrive in the evening, when the slaughter chain is not working.

From the point of view of animal welfare, changes in blood profile indicated that pigs subjected to 3 h lairage did not evidence more adverse consequences on biological parameters related to stress than did pigs who were immediately sacrificed. Changes were seen mostly in pigs subjected to 9 h lairage.

Cortisol levels were high in all the groups in comparison with normal levels for the species as indicated in the bibliography (10–30 ng/mL), indicating a stress response activation [6]. Animals slaughtered immediately upon arrival showed the highest levels probably due to the strong stress effect of loading, transport and unloading. These handling practises involve considerable stress for pigs [2, 5, 23]. The animals given 3 h lairage showed the lowest cortisol levels. Warris et al. [29] found that the recovery of cortisol was complete within 2–3 h, but in other works, Warris et al. [30] found lower cortisol levels in pigs held and fed in lairage overnight, as did Moss and Robb [22]. On the contrary, in our study a tendency to increase can be seen when lairage time is prolonged to nine hours. These differences with other authors may be attributed to fasting.

It is worth mentioning that pigs which are not carriers of stress susceptibility genetic mutation, presented the lowest cortisol levels, indicating a better stress adaptation. Stress-susceptible pigs probably have more difficulty coping with new environment stimuli during transport and lairage.

Serum glucose concentration decreases as lairage time increases, probably as an effect of a prolonged fasting time. In the

absence of feeding, adrenaline stimulates lipolysis. In pigs, fat mobilisation starts after about 16 h of fasting, as an alternative to glucose as a fuel for muscle metabolism and at this point, blood glucose levels would start to recover [17]. In our study, animals in 9 h lairage were in 22 h fasting and blood glucose levels were not recovering. This hypoglycaemia would make the animals feel weak, lethargic and sensitive to the cold [17].

The WBC increased during transportation with respect to the normal values of the species ($11–22 \times 10^3/\mu L$) [19], and did not recover during lairage. This leukocytosis may be caused by the endogenous release of corticosteroids and epinephrine during transport. In acutely stressed animals, a transient leukocytosis is evident within minutes of epinephrine secretion whereas corticosteroid induced changes were not seen until a few hours later. Leukocytosis with neutrophilia, lymphopenia and eosinopenia, observed at 3 h and 9 h lairage, is a typical response to corticosteroids [6, 19].

RBC number was also increased in the three groups with respect to the normal values of the species ($5–8 \times 10^6/\mu L$) [19] which may be attributable to the release of cathecolamines and splenic contraction as a consequence of the acute stress of loading, transport and unloading. The significant increase in RBC in the 9 h lairage group may imply that long periods in the lairage pen without food is a new stress stimulus.

This hypothesis is reinforced by the increase in blood enzymatic activity of CK, LDH, AST and ALT also observed as the lairage time increases. This increase may be associated with physical stress and muscle damage [25], which was more evident in stress susceptible pigs. This damage did not seem to be associated with an increase in physical activity, since a tendency to rest was already evident as lairage time increased from one hour to three hours, when most agonistic interactions usually take place [15]. This might be associated to changes in muscle membrane permeability as a consequence of stress [25]. Other authors [3, 31] have also observed CK and LDH activity increases after transport and lairage. On the contrary, Warriss et al. [30] found lower CK and lactate levels in pigs having rested overnight than in pigs having rested for 1 or 3 h. In our study, lairage time did not have a significant effect on lactate concentration.

From the point of view of meat quality in commercial conditions, a lairage period of more than 3 h would be recommended in order to obtain better ultimate pH values (5.6–6). The mean pH_{24} value of the group of pigs which were killed immediately after arrival was very close to the actual PSE meat pH values both in LT (5.58 ± 0.06) and SM (5.54 ± 0.06), thus showing a tendency to PSE. This tendency is clear when regarding a percentage of pigs with low pH_{24} and high FOP_{24} values in this group (24%). This result is in concordance with the results of other authors [11, 18, 27, 30]. Nielsen [24] observed that feeding pigs on the morning of the day of slaughter could increase the prevalence of PSE, especially if they were slaughtered as soon as they arrived at the abattoir, but in our study, the animals had been fasting for 12 h and in spite of that they showed low pH_{24} values. Pigs subjected to 9 h lairage showed the highest pH_{24} mean. Although this mean value was within the normal range, an increase in the percentage of pigs with high pH_{24} and low FOP_{24} was evident in this group (20%). Some authors have also found a tendency towards DFD meat in long lairage times [11]. Long fasting periods may decrease the glycogen stores and may be one of the causes of DFD incidence combined with stress of handling [17]. Food deprivation for 16–24 h before delivery was recommended by Eikelenboom et al. [9], although De Smet et al. [7] did not find indicators of a positive effect of overnight feed withdrawal on PSE related traits. Based on our results, a rest period might be recommended for

preventing PSE meat but too much lairage time may bring about the opposite situation. Although no significant skin damages were observed in any group, it is worth mentioning the tendency to increase skin damage as lairage time increases, which would mean a carcass quality impairment. This tendency has also been observed by Geverink et al. [14].

We could conclude on the basis of the results obtained from this pilot study, that in Spanish commercial conditions, from the point of view of animal welfare and meat quality, the most adequate pre-slaughter lairage time was three hours for short travel and at a warm environmental temperature. No lairage or excessively long lairage periods without food intake may compromise animal welfare and meat quality. Pigs sacrificed immediately on arrival presented the highest cortisol and glucose levels and a high percentage of PSE meat. A long lairage period without food intake produced hypoglycemia and important muscle damage signs with an increase in percentage of pigs with DFD characteristics. Pigs resting 3 h showed a decrease in cortisol and glucose and no more muscle damage signs than pigs sacrificed as soon as they arrived at the abattoir, and it could prevent PSE and DFD meat. It would be advisable to avoid no lairage or long lairage periods in abattoirs, but a more extensive investigation with replicated studies would be necessary in order to confirm this results and delimit the optimum lairage period.

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