

Reduction of transport-induced stress on finishing pigs by increasing lairage time at the slaughter house

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Summary

Objective: Since pre-slaughter handling such as transport and lairage has been identified as one of the most stressful periods in the pig's life, the main aim of this project was to determine if an increase in lairage time reduces stress markers when the animal transportation period is short. The effect of gender (gilts versus barrows) was also estimated.

Materials and methods: A total of 200 pigs (129 gilts and 71 barrows) were subjected to two different lairage times at the slaughterhouse, short lairage (3 hours) and long lairage (12 hours), after a short journey

(15 km, 20 minutes) by road from farm to abattoir. Blood samples were collected at the beginning of the slaughter line to determine stress markers: acute phase proteins (haptoglobin, serum amyloid A, and C-reactive protein) and neutrophil to lymphocyte (N:L) ratio. In the chilling tunnel, pH was measured in the semimembranosus muscle 45 minutes post mortem.

Results: Our results showed that a longer lairage time allowed the pigs to recover from transport stress, indicated by a decrease in acute phase protein levels (haptoglobin, C-reactive protein), and N:L ratio. Sensitivity to stress did not differ between gilts and barrows.

Implications: Under the conditions of this study, several stress indicators increase less when market gilts and barrows transported a short distance are allowed a longer lairage time (12 versus 3 hours). These stress indicators may be useful measures in research about transport welfare.

Keywords: swine, acute phase proteins, lairage, neutrophil to lymphocyte ratio, gender

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Resumen - Reducción del estrés inducido por el transporte en cerdos de finalización al incrementar el tiempo de estabulación temporal en el matadero

Objetivo: Debido a que el manejo antes del sacrificio tal como el transporte y la estabulación temporal se han identificado como uno de los periodos más estresantes en la vida del cerdo, el principal objetivo de este proyecto fue determinar si un incremento en el tiempo de estabulación temporal reduce los marcadores de estrés cuando el periodo de transportación del animal es corto. El efecto del género (hembras contra machos castrados) también fue evaluado.

Materiales y métodos: Un total de 200 cerdos (129 hembras y 71 machos) fueron sometidos a dos periodos diferentes de estabulación temporal, estabulación corta

(3 horas) y estabulación larga (12 horas), después de un viaje corto (15 km, 20 minutos) por carretera de granja a matadero. Se recolectaron muestras de sangre al inicio de la línea de sacrificio para determinar los marcadores de estrés: proteínas de fase aguda (haptoglobina, amiloide A en el suero, y proteína reactiva-C) y la proporción de neutrófilos a linfocitos (N:L). En el túnel de enfriamiento, 45 minutos post mortem, se midió el pH en el músculo semimembranoso.

Resultados: Nuestros resultados mostraron que un mayor tiempo de estabulación temporal permitió a los cerdos recuperarse del estrés de transporte, indicado por una reducción en los niveles de proteína de fase aguda (haptoglobina y proteína reactiva-C), y la proporción de N:L. La sensibilidad al estrés no difirió entre hembras y machos castrados.

Implicaciones: Bajo las condiciones de este estudio, varios indicadores de estrés disminuyen cuando a las hembras y machos castrados de mercado, transportados por una distancia corta, se les permite un mayor tiempo de estabulación temporal (12 horas contra 3). Estos indicadores de estrés pueden ser medida útiles en la investigación del bienestar de transporte.

Résumé - Diminution du stress relié au transport sur les porcs en finition par augmentation du temps d'hébergement à l'abattoir

Objectif: Étant donné que la manipulation pré-abattage, incluant le transport et l'hébergement, a été identifiée comme une des périodes les plus stressantes dans la vie d'un porc, l'objectif principal du présent projet était de déterminer si une augmentation du temps d'hébergement diminuait les marqueurs de stress lorsque la période de transport des animaux est courte. L'effet du sexe (cochettes vs castrats) a également été estimé.

Matériels et méthodes: Un total de 200 porcs (129 cochettes et 71 castrats) ont été soumis à deux temps d'hébergement différents à l'abattoir, un hébergement court (3 heures) et un hébergement long (12 heures), après un court transport (15 km, 20 minutes) sur route entre la ferme et l'abattoir. Des

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échantillons sanguins ont été prélevés au début de la chaîne d'abattage afin de déterminer les marqueurs de stress: les protéines de phase aiguë (haptoglobine, amyloïde sérique A, et protéine C-réactive) ainsi que le ration neutrophiles/lymphocytes (N:L). Dans le tunnel de refroidissement, le pH a été mesuré dans le muscle semi-membraneux 45 minutes post-mortem.

Résultats: Les résultats ont montré qu'un temps plus long d'hébergement a permis aux porcs de récupérer du stress du transport, tel qu'indiqué par la réduction des dosages des protéines de phase aiguë (haptoglobine, protéine C-réactive) et le ratio N:L. La sensibilité au stress ne différait pas entre les sexes.

Implications: Dans les conditions expérimentales de la présente étude, plusieurs indicateurs de stress augmentèrent moins lorsque les cochettes et les castrats transportés sur des courtes distances furent soumis à un temps d'hébergement plus long (12 vs 3 heures). Ces indicateurs de stress pourraient être utiles lors de recherche sur le bien-être lors du transport.

The welfare of livestock at the farm, during transportation, and at slaughter is an increasing concern in society. Animals can be stressed as much physically as psychologically.¹⁻³ Therefore, in order to obtain an accurate measurement of animal welfare, it is better to use several stress markers simultaneously.

Recently, it has been suggested that acute phase proteins (APPs) may be useful in assessing animal welfare in the short term.^{4,5} These are a heterogeneous group of plasma proteins synthesized in the liver, whose concentration can increase or decrease rapidly (positive or negative APP, respectively) in the presence of stressors such as inflammation and infection. In swine, the main positive APPs are haptoglobin (Hp), serum amyloid A (SAA), C-reactive protein (CRP), and pig major acute phase protein (Pig-MAP). Several studies¹⁻³ have determined the effect of transportation or transportation and lairage before slaughter on APPs in swine.

The neutrophil to lymphocyte (N:L) ratio was selected as a more appropriate marker in the medium to long term.⁶ Several studies across a number of species have shown that stress conditions result in a redistribution of white blood cells involved in the defence and immunological response to antigens, including an increase in neutrophils and a

decrease in lymphocytes, and, as a result, a higher N:L ratio.⁷⁻¹⁰ However we found no studies investigating lairage conditions in which the N:L ratio was used as a stress marker in finishing pigs.

In addition, the degree of stress in an animal is directly related to productivity and meat quality.^{1,2,11} Pigs are among the species most sensitive to stress.² The meat industry has developed several methods to assess meat quality, including pH measures, which are fast, easy to apply, and economical, do not destroy the carcass, and can be used to indicate the stress suffered by the animal.^{11,12} Among the most important influences on final meat quality are transportation, lairage conditions, and genotype.^{2,9}

Pre-slaughter handling has been identified as one of the most stressful periods in a pig's life. In fact, transportation is inherently stressful for these animals and is generally aggravated by unavoidable circumstances such as loading and unloading, vibration, coping with a new environment, restricted space, mixing with other animals, ventilation, and food deprivation.¹ All of these factors can be highly stressful and compromise welfare.¹³ Therefore, optimal transportation and slaughter conditions are essential in order to obtain high-quality meat. There is presently a large body of legislation on these subjects in the European Union.¹⁴⁻¹⁶

The main aim of this project was to determine if an increase in lairage time (from 3 hours to 12 hours) improves stress markers (APPs, N:L ratio, and semimembranosus muscle pH) when the journey length is short (15 km). The gender effect (gilts versus barrows) was also investigated.

Materials and methods

The Bioethical Committee on Animal Experimentation of the University of Murcia approved this trial.

Experimental design

The experiment was conducted in Murcia (southeast area of Spain). The climate of this region is typically semi-arid, and maximum temperatures of 33°C were reached in July, the month in which the animals were marketed. In the first step, Pietrain × (Landrace × Large White) gilts and barrows were reared from April to July (2008) under the same controlled conditions in two adjoining barns belonging to a large commercial piggery. The animals were housed

in fully slatted pens from 20 kg to 100 kg weight (finishing period). Pen size was 3 m × 3 m, with 12 pigs per pen, illuminated by overhead fluorescent lighting (30 lux), and cooled by manually operated water sprinklers when the temperature exceeded 28°C. Animals were fed ad libitum with a pelleted standard feed for growing pigs. Water was supplied ad libitum.

When the pigs weighed approximately 100 kg, they were transported by road from the farm to the abattoir, a distance of approximately 15 km (approximately 20 minutes journey) in a truck with 0.42 m² of space allocated per animal. Feed was withheld for 24 hours before slaughter. The pigs were transported on the 17th of July, at different times for each barn. The first group of animals, coming from one barn, were loaded at 6:00 AM, arrived at the abattoir at 6:20 AM, and had a lairage of 3 hours (treatment short lairage = 3 hours; SL). The second group, coming from the second barn, were loaded at 8:00 PM and had a lairage of 12 hours (treatment long lairage = 12 hours; LL). The temperature for both journeys was similar: 25°C and 28°C at 6:00 AM and 8:00 PM, respectively. Scheduling of the journey was restricted by the slaughter time, which always occurs in the early morning at commercial abattoirs. Sprinklers were not used at the abattoir for the animals transported in the evening. The animals were slaughtered between 9:00 AM and 10:00 AM. When the pigs arrived at the abattoir, we selected the first 100 animals to leave the truck in each treatment (SL and LL), then divided them into two subgroups of 50 animals and allocated them in adjoining rest areas with an available surface of 14 × 2 m². Animals were inspected by veterinarians during the rest periods for signs of disease and injuries.

After the animals were stunned with CO₂, blood samples were collected at the beginning of the slaughter line during exsanguination. The time in the slaughter line was 30 minutes, after which the carcasses were moved to the chilling tunnel (-6°C) for 90 minutes before transfer to cold rooms (5°C) until the meat was dispatched to retail outlets.

Measurements

Two tubes of blood were collected from each animal during exsanguination. One tube, that contained anticoagulant (EDTA), was maintained at 4°C to determine the N:L

ratio. The remaining anticoagulated blood was frozen (-20°C) and used to determine the halothane allele.¹⁷ Animals that were carriers of the recessive halothane allele were eliminated from the study. From the second tube, that contained no anticoagulant, serum was obtained by spontaneous clotting and separation and was frozen at -20°C for APP analysis.

The pH in the right semimembranosus muscle was measured in the chilling tunnel 45 minutes post mortem (pH₄₅) with a hand-held IQ160 pH meter (IQ Scientific Instruments, Inc; Carlsbad, California). The carcasses were weighed and evaluated at the end of the slaughter line.

The N:L ratio was determined for 30 animals per treatment (15 gilts and 15 barrows) randomly selected using a random number generator. Differential leukocyte counts (lymphocytes and neutrophils) were made by microscopic examination of blood smears stained using the May-Grünwald-Giemsa method. Approximately 100 cells were counted and the N:L ratio was calculated. Acute phase proteins were assayed in the same 30 animals per treatment (15 gilts and 15 barrows), including CRP, SAA, and Hp. The proteins CRP and SAA were determined by sandwich ELISA test kits (Porcine C-Reactive Protein Assay and Tridelta Phase Range Serum Amyloid A Assay; Tridelta Development Limited, Ireland). Haptoglobin was assayed using a commercially available spectrophotometric kit (Tridelta Development Limited).

Data analysis

The data was analyzed using the statistical program SPSS 17.0 (Statistical Package for Social Sciences 17, Inc, Chicago, Illinois). The pH measurements were compared with a general linear model (GLM) procedure in which the fixed effects of lairage (SL versus LL) and gender (gilt versus barrow) were included. The interaction between lairage and gender was not included in the model because it was not significant in a previous analysis. Since the N:L ratio and APP were not normally distributed, they were compared using the nonparametric Mann-Whitney U test and median comparison (hypothesis of equal medians), in which the effects of lairage and gender were analysed separately and the interaction could not be studied. In this case, when the effect of lairage was analyzed, it was balanced for gender (ie, each lairage category included half

gilts and half barrows) and when the effect of gender was analyzed, it was balanced for lairage (ie, each gender category included half SL and half LL). Differences were considered significant at $P < .05$.

Results

No signs of disease or injuries were detected when the animals were inspected. Seventeen animals that were carriers of the recessive halothane allele were eliminated from the study.

The effect of lairage time on Hp, CRP, and N:L ratio was significant. Median haptoglobin and CRP were more than twice as high after a short lairage (Table 1), demonstrating that these proteins may indicate early stress. However, median SAA concentration did not differ significantly between SL and LL. Although we observed a high variability, this was the most asymmetric APP. For example, in SL, there was a very great difference between the mean and median (Table 1), with SAA concentration > 100 µg per mL in three of 30 animals and one of 30 animals for SL and LL, respectively.

The N:L ratio differed significantly by lairage (Table 1), with a higher coefficient for SL.

The pH₄₅ of meat was significantly greater for SL than for LL (Table 2). In any case the difference was small, and the pH was high in both treatments, with a pH₄₅ value < 6 in only 6% and 1% of the animals in LL and SL, respectively (data not shown).

No significant differences were observed between gilts and barrows for APP, N:L ratio (Table 3), or pH₄₅ (Table 2).

Discussion

In agreement with our results, several studies in pigs confirm that stress during transport results in an increase in APPs¹⁻³ and a modification of white cell differential counts.^{9,10} Piñeiro et al¹ found increases in Hp, SAA, CRP, and Pig-MAP after a long truck ride (12 hours). Saco et al² observed that Hp and Pig-MAP increased after a long truck ride (6 hours) even with a long lairage time (14 hours). Morley and Kushner¹⁸ found that baseline levels of APPs may be increased up to 1000-fold in sick animals. In agreement with Morley and Kushner,¹⁸ SAA in our study was highly variable and very high concentrations were observed in some animals, highlighting that this APP may increase greatly under stress conditions. Salamano et al³ found that Hp, CRP, Pig-MAP, and N:L ratio decreased when pigs were housed in the abattoir for 28 days. In our study, values of the N:L ratio were higher in animals subjected to SL, indicating a high level of stress as reported by Gross and Siegel.¹⁹ Averós et al⁹ and Sutherland et al¹⁰ also found that the N:L ratio was higher after weaned pigs were unloaded after transport than before loading. Pérez et al²⁰ reported neutrophilia in finishing pigs after transport for 3 hours.

Table 1: Acute phase proteins haptoglobin (Hp), C-reactive protein (CRP), and serum amyloid A (SAA) and neutrophil:lymphocyte (N:L) ratio immediately post slaughter of pigs after a short journey (20 minutes) and either a long (LL) or short (SL) lairage period*

	Lairage	Mean	SEM†	Median	P
Hp (mg/mL)	LL	1.13	0.19	0.79 ^a	< .001
	SL	2.61	0.11	2.82 ^b	
CRP (µg/mL)	LL	22.6	3.43	18.4 ^a	< .001
	SL	58.9	7.49	51.3 ^b	
SAA (µg/mL)	LL	13.6	2.35	11.8	.97
	SL	23.5	5.50	9.30	
N:L	LL	0.61	0.03	0.54 ^a	< .001
	SL	0.81	0.04	0.85 ^b	

* Long lairage, 12 hours (n = 30; 15 barrows, 15 gilts); short lairage 3 hours (n = 30; 15 barrows, 15 gilts).

† SEM = standard error of the mean.

^{ab} For each parameter, medians with different superscripts differ significantly ($P < .05$; Mann-Whitney U test and comparison of medians).

Table 2: Overall mean and least squares differences of meat pH at 45 minutes post slaughter of pigs after a short journey (20 minutes) and either a long (LL) or short (SL) lairage period

	pH	SEM*	P†
Overall mean	6.33	0.05	NA
Lairage effect‡	-0.13	0.04	< .001
Gender effect§	0.06	0.04	.096

* SEM = standard error of the mean or the difference as applicable.

† General linear model procedure in which the fixed effects of lairage (SL versus LL) and gender (gilt versus barrow) were included. Differences were considered statistically significant at $P < .05$.

‡ Long lairage, 12 hours (n = 92); short lairage 3 hours (n = 91).

§ Gender effect: female (n = 118 gilts); male (n = 65 barrows).

NA = not applicable.

Table 3: Acute phase proteins haptoglobin (Hp), C-reactive protein (CRP), and serum amyloid A (SAA) and neutrophil:lymphocyte (N:L) ratio immediately post slaughter of gilts and barrows after a short journey (20 minutes)

	Gender*	Mean	SEM†	Median	P‡
Hp (mg/mL)	F	1.98	0.19	2.22	.62
	CM	2.05	0.22	2.60	
CRP (µg/mL)	F	43.8	6.50	23.2	.82
	CM	44.2	6.80	24.5	
SAA (µg/mL)	F	23.2	6.25	12.3	.09
	CM	13.9	5.95	8.65	
N:L	F	0.69	0.31	0.60	.55
	CM	0.73	0.56	0.66	

* Gender: gilts (F; n = 30; 15 Short Lairage, 15 Long Lairage); barrows (CM; n = 30; 15 Short Lairage, 15 Long Lairage)

† SEM = standard error of the mean.

‡ Mann-Whitney pair and comparison of medians ($P < .05$).

To the best of our knowledge, no scientific literature investigates the effect of lairage, independent of transport time, on APP concentrations or N:L ratio in finishing pigs. However, there are reports in other species that long lairage was associated with less transportation stress. Liste et al¹¹ studied the effect of lairage when rabbits were subjected to short journeys (3 hours). They determined that, with the exception of hematocrit levels, values of plasma stress indicators (corticosterone, glucose, lactate, and creatine phosphokinase) were lower after a long lairage (8 hours) than after a short lairage (2 hours). On the other hand, Tadich et al²¹ found no beneficial effect on the welfare of Friesian-cross steers provided with a long lairage period at the abattoir.

Taking into account all these considerations, our results suggest that APPs may be useful for assessing animal welfare during transport and herd management.¹³ However, the N:L ratio is an optional, economical, and easy to measure indicator of stress in pigs.^{7,9}

We observed lower muscle pH₄₅ values in pigs provided with a long lairage period. This was unexpected, since lower pH₄₅ is an indicator of greater stress. However, for both long and short lairage times, the pH value was within normal range. Aaslyng and Gade²² found no significant effect of lairage time on muscle pH at 40 minutes post mortem in a study that included three lairage times (30, 90, and 150 minutes) after a short journey (1 hour). Further research is needed to define the effect of time in lairage on muscle pH.

The lack of an effect on muscle pH₄₅, which is considered a stress marker for initial meat quality, does not indicate that animal welfare is optimal during the slaughter process. Probably the level of stress required to decrease pH₄₅ is greater than that required to affect stress indicators in the blood.

In this study, we found no significant differences between gilts and barrows in APPs, N:L ratio, or pH₄₅. Other authors^{9,10} did not find a gender effect on N:L ratio response of weaned pigs to transport. However, Piñeiro et al¹ found that CRP and Hp concentrations were significantly higher in gilts than in males. Also, Pérez et al²⁰ found that gilts were more sensitive to physical stress than males, when blood parameters (hemoglobin and glucose) were analyzed. In addition to this result, Pérez et al²⁰ found that pH in the semimembranosus muscle 2 hours post mortem was higher in males, indicating a quicker pH decrease in gilts. In contrast, in other studies, pH was analyzed 24 hours post mortem. Guàrdia et al¹² showed that the probability that gilts and barrows would produce dark, firm, and dry meat (one of the main meat quality defects in pork), was approximately 7% higher than in boars because of their higher final meat pH readings. Also, van der Wal et al²³ observed that aggressive behaviour during lairage occurred more frequently among boars than gilts, and under short stressful periods, gilts were more affected by the stress, resulting in poor quality meat. In agreement with our results, Oliver et al²⁴ and Armero et al²⁵ did not find significant differences among gilts, barrows, or boars for some measurements of meat quality, including pH. Therefore, future studies are needed to analyze the gender effect on different stress markers.

A period in lairage allows animals to recover from the stress of previous handling, which is expected to improve meat quality. We found that 3 hours lairage was insufficient to fully acclimatize pigs, while 12 hours resulted in lower stress measurements. However, as this study did not test an intermediate time between 3 and 12 hours, future work is necessary to test intermediate lairage times. Thus, optimal lairage time can improve animal welfare and, therefore, increase the ethical quality of the meat, ie, by minimizing unnecessary animal suffering.¹¹

Implications

- Under the conditions of our study, several stress indicators (serum Hp and CRP levels and the N:L ratio) increase less when market gilts and barrows transported a short distance (15 km) are allowed a longer lairage time (12 versus 3 hours).
- These stress indicators may be useful measures in transport welfare research.

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