Influence of Pre-Slaughter Process on Pork Quality:
An Overview

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Handling practices prior to slaughter have significant influence in the stress level and animal welfare of pigs, and consequently, in the final meat quality. This paper reviews current knowledge about ante mortem factors (fasting period, farm handling, mixing, loading, unloading, transport, lairage conditions, and driving to the stunner) related to the live animal that influence pork quality with special emphasis on technological quality attributes. The development in slaughter technologies and their practical applications in pig slaughterhouses are described. In spite of genetic effects and slaughter techniques, poor pre-slaughter operations lead frequently to an increase of physiological and physical stress in pigs. Ante mortem factors, such as lairage time or moving pigs into the slaughter rooms, and inadequate design of slaughterhouse facilities have an important effect on pig stress, affecting meat quality after slaughter. Some recommendations to guarantee animal welfare and obtain the best meat quality are reviewed.

Keywords pig handling, animal welfare, abattoir design, meat quality

Introduction

Pork quality is affected by several factors, including genetics (frequency of Halothane and RN genes in certain breeds or strains, and polygenic effects), pre-slaughter handling (farm handling, fasting, mixing, loading, transport, lairage conditions and driving to the stunner) and slaughter procedure (method of stunning, slaughter, scalding-dehairing, and method and time of chilling after slaughter). This paper reviews current knowledge about factors of importance for pork quality with special emphasis on technological quality attributes related to pre-slaughter handling.

It is evident that genetics is an important factor that influences pork quality and comprises differences among breeds. These differences can be caused by small differences in a large number of genes (polygenic effects) and also, with major genes like Halothane and RN genes. The Halothane gene is related with the porcine stress syndrome (PSS) gene and causes malignant hyperthermia, hyperpyrexia, metabolic acidosis and an increase of muscle rigidity in pigs exposed to the anesthetic gas halothane. It has also been associated with development of PSE (Pale Soft Exudative) meat. The RN gene is associated with reduced technological yield (overall quality and yield) and leaner
carcasses, high muscle glycogen stores and an extended post mortem decline in pH. Meat obtained from these pigs is often referred to as acid meat due to the low pH. The heritability of most attributes referring to the quality of pork, such as intramuscular fat content, is related to polygenic effects. During the last 50 years, intensive selection for muscle development and fat reduction has contributed to the increased incidence of PSS in live pigs and the PSE condition in meats. However, only 4% of inferior meat quality is due to genetics with the remainder being due to pre-slaughter and post-slaughter treatment. In fact, the tendency in meat science is to establish the existence of a new genetic pool in the commercial pig populations of tomorrow, free of the Halothane and RN genes, where the influence of slaughter factors on pork quality may be fundamentally different.

The importance of these slaughter factors on pork quality has been revealed in most recent studies. Some studies concluded that improper pre-slaughter handling leads to unfavorable pH and temperature in meat of stressed pigs, normally giving rise to inferior meat quality (e.g., low water holding capacity-WHC), even in pig populations free of the Halothane gene. Other studies revealed that processing plants might cause variation in meat quality independent of pre-slaughter factors (e.g., genetic background of animals). With the exception of the above-mentioned genetic factors, it is well known that the treatment of animals prior to slaughter can lead to a set of irreversible deficiencies in meat quality and derived products. Even the treatment of pigs during slaughter can produce physiological and behavioral changes in animals, which are unused to being manhandled. The effect of the stress-induced physiological changes on post mortem processes involving meat quality differs between the muscle metabolisms of different animals. Indeed, knowledge of handling techniques allows greater control to be exercised over all variables that can affect meat quality. The current review outlines the present knowledge of significant pre-slaughter factors affecting technological meat quality.

Fasting

According to the review of the animals welfare during transport made by an expert group of the Scientific Committee on Animal Health and Animal Welfare, pigs should be fasted no more than 10 h before collection and transport. However, feed withdrawal in pigs from 12 to 18 h is a common practice in several countries to decrease mortality rate during transportation and lairage, and to also decrease the associated problems with manipulation of full intestines in slaughterhouses. This period of feed restriction reduces the risk of microbial cross-contamination during the slaughter process. Moreover, the effects of behavioral and physiological stress responses on muscle metabolism depend on the energy status of the pigs. So, fasted pigs in comparison with fed pigs have much more aggressive behavior and show an acute decrease of muscle glycogen during fighting. When the fasted pigs are subjected to extended transportation and lairage, increased aggressiveness and fighting cause an increase in skin damage and lacerations, and a decrease in carcass yield. On the other hand, death during transportation is higher in recently-fed animals due to the pressure exerted by the full stomach on the vena cava, which reduces its diameter and worsens the circulation of blood. This process is often aggravated by the animal inhaling its own vomit. Also, it increases the risk of spread of Salmonella at evisceration. Consequently, slaughter procedures are likely to have a larger effect on ante- and post-mortem metabolism in fasted than fed pigs.

The duration of feed withdrawal is also a parameter worth consideration because of both its relation to the extent of the pH drop in the meat of slaughtered animals and its possible implications for the development of PSE meat. It is widely accepted that a fasting
period helps to reduce most of the carbohydrates available for post-mortem conversion of glycogen into lactic acid, reducing muscle glycogen stores in pigs during slaughter, increasing final pH ($pH_{24}$), and thereby improving water holding capacity (WHC) and color. However, the increase of $pH_{24}$ observed after a fast period up to 24 h is not enough to be classified as DFD (Dark Firm Dry) meat.\(^{(24)}\) The risk of DFD and PSE conditions according to on-farm fasting time was considered in different studies.\(^{(25, 26)}\) According with these authors, the lowest risk of DFD and PSE conditions was achieved with on-farm fasting times between 14–22 h and 18–22 h, respectively, with values around 17% and 7% for DFD and serious DFD defects, and values around 40% and 4% for PSE and serious PSE defects, respectively. However, the slight differences of PSE and DFD risks observed in the whole farm fasting time studied (between 4 and 34 h) for each PSE and DFD conditions (serious and moderate) suggest a non-significant development of these defects between low and high fasting periods. Although on-farm feed withdrawal from 12–18 h before delivery is a good practice for preparing pigs for transportation\(^{(14, 27)}\) findings concerning the optimum period of withdrawal are very contradictory and the advantages of feed restriction are not unanimous in the literature (Table 1). Some studies conclude that it is necessary for at least 12 h\(^{(20)}\) of fasting while others suggest more than 24 h\(^{(28, 29)}\) is needed to observe a significant decrease of PSE meat. In other studies, the relationship between feed withdrawal and incidence of PSE and DFD meats when feed restriction was prolonged during the night before slaughter\(^{(30)}\) or exceeded 72 h\(^{(31, 32)}\) was not detected. Following this, many authors have noted significant reductions in the quality of meat from pigs that have been fed before being taken to slaughter, particularly in hot weather and with stress-susceptible pigs (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Fasting time</th>
<th>Observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>High risk of vomit during transport</td>
<td>111</td>
</tr>
<tr>
<td>2–6 h</td>
<td>Much higher mortality rate in transported pigs</td>
<td>72</td>
</tr>
<tr>
<td>4–12 h before loading</td>
<td>Appropriate period to feed withdrawal</td>
<td>21</td>
</tr>
<tr>
<td>6–12 h</td>
<td>Low mortality rate in transported pigs</td>
<td>72</td>
</tr>
<tr>
<td>Feed before transport</td>
<td>Aversion to transport was greater</td>
<td>112</td>
</tr>
<tr>
<td>Feed the day of transport</td>
<td>Death losses are greater</td>
<td>80</td>
</tr>
<tr>
<td>Overnight fasting</td>
<td>Increased ultimate meat pH, meat color and WHC</td>
<td>113</td>
</tr>
<tr>
<td>Overnight feed withdrawal</td>
<td>No effect on meat quality (PSE)</td>
<td>30</td>
</tr>
<tr>
<td>9–18 h</td>
<td>Increase of losses weight in carcass</td>
<td>54</td>
</tr>
<tr>
<td>&lt;12 h or &gt;18 h</td>
<td>Increase in total mortality (transport and lairage) and higher physiological response (cortisol) in pigs</td>
<td>13</td>
</tr>
<tr>
<td>16–24 h</td>
<td>Advantageous to the packer</td>
<td>85</td>
</tr>
<tr>
<td>24–48 h</td>
<td>Reduction of PSE meat in stress susceptible pigs</td>
<td>29</td>
</tr>
<tr>
<td>&gt;72 h</td>
<td>No relationship with ultimate quality ($pH_{24}$)</td>
<td>32</td>
</tr>
<tr>
<td>&gt;96 h</td>
<td>Increase of 8% in the incidence of DFD pork</td>
<td>114</td>
</tr>
</tbody>
</table>

WHC: water holding capacity; PSE: pale soft exudative; DFD: dark firm dry; and $pH_{24}$: pH at 24 h post mortem.
Pre-Slaughter Handling

It has long been recognized that negative handling or moving of pigs prior to slaughter results in high stress and can adversely affect the animal welfare and pork meat quality. These handling practices can induce stress either psychologically or physically. Pre-slaughter stress can be divided into long-term stress (farm handling, mixing, loading and transport), mainly associated with the development of DFD meat, and short-term stress (lairage conditions and driving to the stunner), which mainly leads to meat quality associated with PSE condition. Handling and transportation are considered as major stressors for farm animals. A recent study performed to investigate the effects of pre-slaughter conditions on pork quality states that pigs managed according to a chain assurance quality protocol avoiding established risk factors during transport and lairage by the control of parameters such as the driver’s experience, transport time, stocking density on truck, offloading time, group size in lairage, stocking density and lairage time, showed better potential for improved meat quality through the slaughterhouse procedures. However, since detailed assessment of how specific handling procedures affect meat quality are unavailable, it is not known to what extent quality could be influenced by poor handling, or genetic variations.

Pre-Transport Handling

One stressful operation on the farm is driving pigs from the fattening pen to the truck. Poor on-farm handling of these operations increases the susceptibility to pre-slaughter stress, and pigs react with an inborn fear response that may adversely affect welfare, productivity, and ultimately meat quality. Negative handling causes a high stress and hormonal response, such as a mechanism of innate defense, resulting in an increased heart beat rate, arterial pressure, and a high mobilization of nutrients toward the blood (Fig. 1). Also, these reactions cause a significant reduction of early post-mortem muscle glycogen amounts and lower pH24, as well as a higher incidence of PSE meat compared with pigs that were handled correctly on the farm. In order to control these blood parameters, the use of electric goads to make pigs move quickly and facilitate the load process should be avoided.

### Table 2

<table>
<thead>
<tr>
<th>Fasting time</th>
<th>Lairage (h)</th>
<th>Meat quality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Since the day before slaughter (12–18 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.8</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>5.8</td>
<td>17.0</td>
</tr>
<tr>
<td>4</td>
<td>1.9</td>
<td>20.2</td>
</tr>
<tr>
<td>Feeding a few hours before slaughter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13.1</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>13.1</td>
<td>10.3</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>6.2</td>
</tr>
<tr>
<td>24</td>
<td>2.5</td>
<td>7.4</td>
</tr>
</tbody>
</table>

PSE: pale soft exudative; and DFD: dark firm dry.

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[28] Influence of fasting duration and lairage time on meat quality of pork.

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[29] Fasting time

<table>
<thead>
<tr>
<th>Lairage (h)</th>
<th>Meat quality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td>5.8</td>
</tr>
<tr>
<td>4</td>
<td>1.9</td>
</tr>
<tr>
<td>24</td>
<td>2.5</td>
</tr>
</tbody>
</table>

PSE DFD
Pigs also should be allowed to rest, unmixed, in the collecting pens before being loaded.\(^{(23)}\) Mixing or regrouping pigs from different familiar groups in the same collecting pen before loading to trucks can increase the excitability level, and the unavoidable fights between animals. The presence of entire males in these groups can increase the problem because these animals frequently fight with more aggressiveness to establish a new dominance order.\(^{(41, 42)}\) The principal consequences of fighting are skin lacerations and damage of the carcass surface, particularly in the shoulder region.\(^{(43, 44)}\) In other cases, fighting can cause major carcass bruising with subsequent financial losses to the meat industry, \(^{(45)}\) principally where good pre-slaughter practices are not exercised. Also, the unaccustomed physical activity during fighting leads to increased blood levels of cortisol and creatine phosphokinase (CPK),\(^{(46)}\) as well as high muscle glycogen depletion, and consequently, a progressive higher ultimate pH and DFD meat condition.\(^{(23, 47)}\) Mixing of unfamiliar pigs led to an increase of PSE condition up to 22%.\(^{(48)}\) However, several studies have shown that pH\(_{45}\) (pH at 45 min) was not affected by fighting between pigs and consequently there should be a low incidence of PSE meat.\(^{(43, 49)}\) Other studies have shown that moving a reduced group of pigs (<15) from the fattening pen to the lorry deck reduces aggression and promotes resting behavior compared to larger groups, even when the groups were composed of mixed pigs.\(^{(50)}\) Although there is little evidence that low stocking densities (high available space per pig) promote fighting in pigs, low stocking density (>0.39 m\(^2\) space/100 kg pig) during transport to prevent easy movement (which encourages fighting) is suggested.\(^{(51)}\) In the abattoir, if mixing animals is unavoidable, the reduction of lairage time (<30 min) and the confinement of the animals in a reduced space might be two ways to reduce aggression.\(^{(52)}\)

Loading animals into trucks on farms and unloading at the abattoir are the main stresses experienced by pigs during transportation. Under unfavorable conditions, both operations can increase the heart rate up to 200 beats/min for several minutes before
stabilizing (Fig. 2), and have a negative influence on meat quality.\textsuperscript{(53)} During loading, the increases in heart rate and body temperature are proportional to the slope of the ramps used and the difficulty involved in negotiating them,\textsuperscript{(54)} and higher physical and emotional stress is seen in the pigs.\textsuperscript{(24)} The lack of light in the trucks is another frequent cause of many animals’ reluctance to be loaded.\textsuperscript{(55, 56)} In optimum conditions, the whole loading process should last less than one hour in order to make it easier and less stressful for pigs.\textsuperscript{(57)} Loading at the farm and off loading at the abattoir can be improved using platforms at the same level as the trucks, hydraulic lifts, permanent ramps at an angle of less than 20\(^\circ\), and portable ramps with a slope of less than 27\(^\circ\).\textsuperscript{(23, 24)} The animals will load more easily through gently-sloped double ramps separated by central panels with solid lateral protection, and a wide design to facilitate groups of 2-3 animals loading at the same time.\textsuperscript{(58)} Otherwise, steep ramps do not encourage the animals to leave the trucks voluntarily and are often incompatible with concerns for their protection and safety. Some authors note that if these factors are not borne in mind, an inevitable increase in injuries and physical harm to the animals can occur.\textsuperscript{(23)} More shoulder lesions and loin lesions are frequently observed in pigs with an offloading time of more than half an hour, increasing with off-loading time.\textsuperscript{(59)} As regards the effects of loading system on muscle metabolism (Table 3), a higher incidence of hemorrhages in the shoulder and greater percentage of PSE meat condition has been reported in pigs loaded by ramps compared to hydraulic lift systems.\textsuperscript{(60)}

\textbf{Transport}

Transportation of pigs to slaughterhouse is considered one of the most important pre-slaughter factors, since even in the best conditions it can cause marked stress or fatigue in animals. As a result, there are frequently losses in live weight,\textsuperscript{(61)} or even deaths (Table 4),

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{heart_rate_evolution.png}
\caption{Evolution of heart rate (mean values) in pigs during short length transports (n = 91) and the \textit{ante mortem} (resting and handling) and slaughter (stunning and bleeding-out) phases. (modified from 120)}
\end{figure}
as well as alterations in post-mortem metabolism with the concomitant deterioration of meat quality.\(^{62, 63}\) This stress may be related to factors such as the distance traveled, type of truck, stocking density, speed and vibration of vehicle, noises, environmental conditions during transportation, contact with unknown people, mixing of animals from different origins, hunger, thirst, and fatigue of pigs.

**Transport Vehicles.** The type of vehicle plays an important role as well as the way the load area is equipped.\(^{54}\) Flexible equipment with barriers reduces the risks of motion sickness and damage as they grant optimal loading density. In addition, dividing the load area in the interior of the vehicle serves to reduce social stress and avoids potential sources of danger in pigs. The style of driving and the conditions of the road surface determine that any start, acceleration, braking or turning exposes the animals to thrusting or centrifugal forces. There is some evidence from studies\(^{42}\) of pigs that vibration associated with transport is likely to have adverse effects. Vibrations during transport are an important cause of animal stress, provoking an increase of heart rate. In general, transportation conditions affect post-mortem meat quality by provoking stress or animal fatigue.\(^{63}\)

**Length of Transport.** Regarding duration of transportation, in general it is thought that long-distance transportation causes greater depletion of muscular glycogen, which causes

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**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>Ramp</th>
<th>Hydraulic lift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pigs</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Shoulder haemorrhages (%)</td>
<td>12.5(^a)</td>
<td>5.8(^b)</td>
</tr>
<tr>
<td>PSE SM* (%) (pH(_1&lt;6.00))</td>
<td>11.8</td>
<td>8.8</td>
</tr>
</tbody>
</table>

\(^{a}\)PSE: pale soft exudative; and SM: semimembranosus muscle. \(^{ab}\)Means with different superscripts are different (P < 0.05).

**Table 4**

<table>
<thead>
<tr>
<th>Country</th>
<th>Transport mortality (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>0.24, 1.0</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>116</td>
</tr>
<tr>
<td>Spain</td>
<td>0.22</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>&lt;0.20</td>
<td>117</td>
</tr>
<tr>
<td>UK</td>
<td>0.07</td>
<td>51</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>0.15, 0.5, 0.21</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>51</td>
</tr>
<tr>
<td>Norway</td>
<td>0.20</td>
<td>118</td>
</tr>
<tr>
<td>Denmark</td>
<td>0.016</td>
<td>119</td>
</tr>
<tr>
<td>Belgium</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>0.16</td>
<td>51</td>
</tr>
<tr>
<td>Italy</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

Regarding duration of transportation, in general it is thought that long-distance transportation causes greater depletion of muscular glycogen, which causes
an increase in final pH ($\text{pH}_{24}$) and greater development of DFD meat.\(^{(64)}\) Other authors have found that pigs transported 650 km showed a significant increase in pH$_{45}$ and pH$_{24}$, and gave darker meat than those transported 180 km.\(^{(62)}\) However, there was no evidence that the long transport periods (1–4 h) affected the incidence of potentially PSE or DFD meat based on measures of pH (no increase in muscular pH$_{24}$), color or drip loss although the *Longissimus dorsi* muscle was slightly darker in pigs transported for 4 h than that of pigs transported for 1 h.\(^{(65)}\) In another study, 8 h of transport was found to improve tenderness, compared to 0.5 h, due to reduced glycolytic potential at the time of slaughter and subsequent higher ultimate pH.\(^{(66)}\) Transportation around 1 h does not lead to important losses of muscular glycogen, so there is not significant development of DFD meat.\(^{(67)}\) In addition, the level of lactic acid accumulated during loading of animals tends to decrease, normalizing the incidence of PSE condition.\(^{(68)}\) So, the impact of transport stress can be reduced by limiting the length of journeys and by ensuring good conditions during transport like an adequate preparation of the animals, controlled prior access to feed and water, minimal disruption to social groups and adequate loading facilities.\(^{(42)}\)

A rationale for restricting journey time could be made on the basis of the criteria that welfare is adversely affected after a specific journey duration, that transportation is a continuous aversive experience for animals, and that if animals were sub-clinically infected with a disease, a restriction of journey time would reduce the distribution of the infectious disease and allow more time for additional disease control measures.\(^{(69)}\) Nevertheless, immediate pig slaughter following short journeys (<1 h) involves loading, transport and unloading all being carried out in a short space of time, and genetically susceptible animals show a greater tendency to become excited. This causes more PSE meat than in pigs transported for longer distances under good conditions.\(^{(48)}\) As seen in Fig. 3, the effect of transportation time on the risk of PSE condition depends on stocking density.\(^{(25)}\) For a high stocking density (0.25 m$^2$/100 kg pig), there is less effect of transportation time and little change in risk of PSE condition between transits of 1–7 h. In comparison, at low stocking density (0.5 m$^2$/100 kg pig), up to a 6% difference in PSE meats was observed between 1 and 7 h transport time. The maximum PSE risk was observed at 1 h of transport (~8%), and the minimum at 7 h (~1%). Fig. 3 also shows that PSE risk increases with the stocking density in the first 4 h of transportation while this behavior changes up to 4 h, observing a decrease in the PSE risk as stocking density increase.

**Load Density.** Another prerequisite to prevent stress during transport is sufficient loading density, which allows animals to lie down. The number of pigs per load is a parameter that influences heat stress development; more fatigue and aggressiveness, lower meat quality and a higher mortality are observed if too little space is allowed. The new legislation in force since January 2007 concerning the loading density (the Council Regulation (EC) n. 1/2005\(^{(70)}\)) recommends a maximum loading density for 100 kg pigs of 235 kg/m$^2$ (0.425 m$^2$/100 kg), in order to guarantee animal welfare during transportation. Higher load densities (321 kg/m$^2$ or 0.31 m$^2$/100 kg) are considered unacceptable for the transport of pigs since it negatively affects the animal’s health and well-being, causing higher levels of physical stress related with a high activity of the enzyme CPK in the blood,\(^{(71)}\) higher mortality rates,\(^{(72)}\) and worsening of meat quality.\(^{(58)}\) Animals transported in small spaces suffer more from fatigue and exhaustion, which is translated into increased pH values both 45 min and 24 h post mortem.\(^{(73)}\) For this reason, when load densities are moderately high (281 kg/m$^2$ or 0.35 m$^2$/100 kg), the journey should be no longer than three hours to minimize the adverse effect on the welfare of pigs.\(^{(71)}\) Some authors observed under environmental conditions that varying stocking densities between 0.35 and 0.50 m$^2$/100 kg had
relatively little effect on blood profile or meat quality for commercial transports less than approximately 3 h journey time. In contrast, a risk of increased skin damage due to trampling and/or fighting was observed when more than 0.35 m²/100 kg was given during transport.

Differences between authors as to the appropriate limits of load density to guarantee the animal welfare and consequent meat quality may reflect regional differences in weather conditions, and the proportion of the local pig population with inherited stress susceptibility. For example, the use of load densities of more than 0.40 m²/100 kg is recommended for the best meat quality indices, and when the area per 100 kg of pig during transport was reduced to less than 0.40 m², a higher transport mortality (0.77%) is observed. Other studies observed that all carcasses showed PSE-characteristics when loading density was around 0.40–0.45 m²/100 kg; whereas, when load density was 0.5 m²/100 kg, only 31.2% and 25% of carcasses showed this reduction of meat quality. In addition, the effect of stocking density on pre-slaughter mortality and ultimate meat quality is greatly influenced by breed and ambient temperature. A low prevalence of the Halothane gene can also lead to reduction in mortality rates during transport and holding times. These authors found in 107 samples of dead pigs during transport and holding times in abattoirs, a 71% of positive homozygous gene (nn), 24% of heterozygous gene (Nn), and only a 4.7% of pigs were free of this mutation (NN).

Environmental Conditions. Higher levels of temperature and humidity in the truck compartment can endanger the animal’s breathing, and mortality can increase at temperatures over 25ºC. Transportation of pigs at over 28ºC favors stress development since the heart and breathing rates are significantly quickened. For this reason, the incidence of PSE carcasses may easily double during the first hour of transportation in hot days. When vehicles are ventilated during transport, the excessive temperature and humidity in the animal compartment can be reduced, improving transport conditions. Furthermore, excess body heat suffered by pigs during loading can produce an increase in the air temperature within the compartments. In addition, when there are stops or prolonged

Figure 3. Risk of PSE pork meat according to stocking density (0.25–0.5 m²/100 kg pig) and transportation time. (modified from 25)
waits before slaughter, the pigs can suffer from phenomena such as “heat stroke,” which produces greater weight loss and worse meat quality,\(^{(84)}\) or even death. The influence of low temperatures on meat quality, though, is negligible.\(^{(85)}\) Several studies show that the best transportation temperature is between 10°C and 15°C, since above 15°C mortality rates increase due to high susceptibility to heat stress and the animal’s inability to dissipate the heat effectively.\(^{(86)}\) These studies found a direct relationship between ambient temperature and mortality during transportation, based on observations in Northern European countries, which showed that mortality increases steadily between 10ºC and 18ºC before rising sharply at higher temperatures. In Southern countries, such as Spain, similar studies did not find such a clear relationship.\(^{(13)}\) On the contrary, higher mortality rates were found in October and November, far cooler months, largely because extreme precautions are taken in the summer, when average temperatures are over 25ºC.

**Lairage**

The rest period of animals prior to slaughter allows them to recover the physiological condition lost during transportation; muscular glycogen returns to normal level, and the animals are more likely to relax. Nevertheless, inadequate treatment of pigs in this stage may result in additional stress and physical pain leading to further deterioration of meat quality. Also, fluctuating temperature and relative humidity during lairage may have an additional effect on meat quality\(^{(87)}\). The practice of spraying pigs with cold water is recommended after transportation and before slaughter in order to clean the surface of the body, reduce aggressiveness and facilitate electrical stunning by lowering skin impedance.\(^{(23)}\) The pens should be adequately ventilated, water *ad libitum* should be supplied, and there should be constant cold showers during the warm seasons,\(^{(82)}\) in order to reduce the muscular temperature before bleeding and achieve close to normal body temperature at the moment of *post mortem* metabolic muscle conversion in meat.\(^{(88)}\) Some authors observed that the application of cold-water showers (~10ºC) caused temperature drops in muscle mass at 40 min *post mortem* sufficient to reduce the incidence of PSE condition.\(^{(89)}\) The body temperature reduction of 2ºC before bleeding out achieves a 37% reduction in the degree of myosin denaturation and therefore, in exudation losses of meat.\(^{(90)}\) Once in the pens, the pigs quickly establish a dominant hierarchy within the group. This brings about increased aggressiveness with an increase of body damage scores, and could be avoided if different batches were not mixed in one pen and if there were enough space for the animals.

**Resting Time.** It seems that those pigs that are slaughtered in the first few hours of penning (<2 h), when aggressiveness is more pronounced, suffer greater physical and physiological stress,\(^{(91)}\) as a result of which there is more metabolic activity, lower muscular pH\(^{(92)}\), higher body temperature, and reduced meat quality\(^{(93)}\). Similarly, a higher incidence of PSE meat (>40%) has been detected when animals from different farms are mixed,\(^{(48)}\) especially when the penning time of the pigs is very short (only 30 min–1 h), or when animals are immediately slaughtered after delivery to the abattoir.\(^{(20)}\) Nevertheless, slaughtering pigs immediately after arrival at the abattoir would reduce skin damage scores in animals mixed in lairage and would reduce the incidence of PSE carcasses at stressful environmental conditions.\(^{(52, 87)}\) Typically, this dominant hierarchy and aggressive behavior in pigs normally ends after about 2 h of penning\(^{(58)}\); although, after 3.5–4 h rest, the animals begin to show aggressive behavior again, especially in males, and again, this endangers meat quality. For this reason, several authors suggest that slaughtering pigs
after 2–4 h rest leads to a significant reduction in the problem of PSE meat. Other authors found that the optimal rest period for pigs to obtain the best meat quality indexes was 3–5 h, 4–6 h, or 6–8 h in pigs sensitive to stress. After more than 9 h of lairage, aggressiveness and fights among the animals are again more likely, which raises stress indicators in the blood [cortisol, lactate and creatine phosphokinase (CPK)] and thus, increases the proportion of skin damage and DFD meat as well as the incidence of PSE meat. However, if the animals are penned at night, even though they are more aggressive, the levels of cortisol, lactate, and CPK are reduced, which means lower stress levels for pigs. Other studies show that the higher PSE incidence found in pigs penned at night was more related to stress caused by being woken up and taken to the restrainer than to the fact of having spent so many hours in lairage during the night. In any case, rest periods longer than 11–12 h cause considerable alterations in the energy reserve of muscular glycogen, increasing the incidence of DFD meat as well as losses in live weight and carcass weight. According to the Scientific Committee on Animal Health and Animal Welfare, a maximum of 8 h after a journey commences pigs should have a rest period of at least 6 h. When a journey has continued for 8 h, plus 6 h rest, plus a further 8 h travel, the pigs should have a rest period of at least 24 h, during which they should have food and water available.

Transfer to Stunning Area. Transfer to the stunning system and restrainer prior to slaughter also influences pigs welfare and could cause an increase in stress level of the pigs. As can be observed in the Figure 2, these operations can increase the heart rate up to 200–250 beats/min, where it remains practically constant during the stunning and part of the bleeding-out phase. Pigs are especially sensitive to the treatment they receive, so the appearance of skin damages and open cuts could occur during immobilization in poor conditions, reducing the carcass quality. The use of sticks and electric prods to guide the animals into the restrainer caused refusal to enter in over 50% of cases, with few pigs entering voluntarily. Indeed, the excessive use of electric goads results in greater stress, higher skin damage scores, and an elevated incidence of PSE meat and blood splashing.

In addition, the task of leading groups of more than 4 pigs required less effort on the part of the slaughterhouse operator than herding 1 or 2 pigs, and the pigs showed less tension and stress. Pigs have no instinct to walk in line, so the journey to the slaughter area has to be as short and as safe as possible, along double corridors in which they are able to see each other, in order to stimulate their herd instinct. The use of automatic push gate systems in plants with CO₂ stunning systems to lead the pigs in small groups to the stunning point, reduces the stress caused by forced human-animal interaction. Pigs develop frequent evidence of fear and excitation with high stress if they are led in a single queue without any mutual visual contact and with frequent interaction with humans. In these cases, the concentration of blood catecholamine and body temperature increases, and the muscle acidification rate is faster than normal handling. This has a negative effect on meat quality parameters (greater PSE meat incidence) when the acute stress suffered before stunning leads to an accelerated decrease in muscular pH as the carcass is still warm.

Research on Pre-Slaughter Stress Assessment

Research on stress during animal handling and transportation indicates that these are major stressors for farm animals and might have deleterious effects on their health, well-being, performance and ultimately on product quality. Frequently during these
stages, there are many difficulties in obtaining information from animals in an environment where space and visibility are very limited. Many of the recent studies are focused on the establishment of noninvasive methods of data collection directly related with the stress response. Acute phase protein testing from blood samples taken at the farm before transport and at the abattoir has been related to lesions and stress caused by different procedures for handling, transport, and lairage.\textsuperscript{(105)} Plasma cortisol and catecholamine responses of animals to transportation might provide useful evidence that transport is an aversive experience to a farm animal.\textsuperscript{(69)} This is based on the assumption that farm animals are capable of experiencing negative emotional states that could be associated with the same types of physiological changes that occur when humans report that they are experiencing distress.\textsuperscript{(106)} Automated recording of stress vocalizations has been proven as a useful non-invasive method for impaired welfare assessment during pig housing and transportation.\textsuperscript{(107)} Another non-invasive method that has been successfully developed and validated is the determination of stress-indicating metabolites in saliva, feces, or urine.\textsuperscript{(108)} A saliva collection device located in the mouth of the animal provides information on stress hormones that have passed through a membrane into a tube from where the average hormone concentration is analyzed.\textsuperscript{(109)} Alternatively, observation methods such as video cameras combined with physiological stress parameters can help to recognize different animal behaviors.\textsuperscript{(110)}

Conclusions

The meat quality response to pig handling in the 24 hours before slaughter is very important in determining the yield of the carcass and the technological quality of meat for the processing industry. There is no doubt that poor environmental conditions during pre-slaughter handling, while the animals are still alive, can irreversibly affect the quality of the meat. Quality can be improved with the knowledge of these processes and guaranteed only through adequate control of different stages before slaughter. However, there are no common recommendations in the literature for ideal time or length of the different pre-slaughter processes for achieving the highest quality and yield of pork meat. Based on the literature results, avoiding mixing of unfamiliar animals, minimizing lairage time, and using good handling practices could reduce the amount of stress, and consequently, meat quality problems related to PSE and DFD conditions. Such measures would also be desirable from an animal welfare point of view, and would reduce the incidence of skin damage.

In general, the recommendations would include feed withdrawal about 12–18 h before transport and lairage to prevent dead losses and improve meat quality. Electric goads should not be used to make pigs move more rapidly and the load process onto the lorry deck should be facilitated within 1 h, for example by using collecting pens near to the lorry, hydraulic lifts, and reduced groups of pigs without mixing. The stress of animals during transport can be reduced by limiting the length of journey (around 1 h), guaranteeing an adequate preparation of animals (prior access to feed and water), a sufficient loading density (a maximum of 0.425 m\textsuperscript{2}/100 kg) and adequate environmental conditions in the lorry (temperature, humidity, ventilation), and by ensuring good transport conditions (i.e., avoiding excess stops or waiting periods) that reduce stress to the animals. Prior to slaughtering, rest periods should minimize aggressive behavior and relax the animals. For that, recommendations include a lairage of 2–4 h, spraying pigs with cold water, adequate ventilation, supplying water \textit{ad libitum} and no mixing pigs of unfamiliar groups. Special care must be taken during the transfer of pigs to the stunning system. These operations represent the main source of stress for pigs, especially when bad conditions are practiced.
Here, sticks or electric prods to guide animals should be avoided and the animals should be guided in reduced groups along double corridors in order to have the best results in terms of carcass and meat quality.

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