Abstract: The incidence of PSE (pale, soft and exudative) pork meat is directly influenced by pre-slaughter handling of animals, compromising meat quality and resulting in economic losses for industry. Considering management, the main extrinsic stress factors are: the pre-slaughter rest time in lairage, the total fasting time and at farm and the transport time. To evaluate the effects of these factors on PSE meat incidence, pH of 1038 carcasses was measured at 45 minutes post mortem (pH45) in a pig slaughterhouse in Paraná, Brazil. Carcasses with pH45≤5.8 were classified as PSE, and those with pH45>5.8 as normal. In order to verify if the factors influence PSE meat appearance, it was divided into categories according to its duration, and according to the number of animals evaluated in each category, the PSE meat frequency was estimated, and which time intervals obtained the lower incidence of the change. The total incidence of PSE carcasses was 19.17%. The periods of rest in lairage, total fasting, fasting at farm and transport that minimized the occurrence of PSE meat were from 04h01 to seven hours, from 14h01 to 17h00, from 03h01 to five hours, and from 02h01 to three hours, respectively.

Key words: fasting time, lairage time, pH45, pre-slaughter handling, transport time, swine.

INTRODUCTION

Brazilian pig farming, in order to comply with the new consumer market requirements, started to produce swine with high production of lean muscle thought the interaction of genetic enhancement, health improvements, nutrition and production systems. Thus, in addition to the desirable increase in lean meat production, there were changes in biochemistry and substantial composition of muscles. These changes responsible for formation of pale, soft and exudative (PSE) meat, were caused by appearance of the Pork Stress Syndrome (PSS). This syndrome is a consequence of a genetic mutation in the calcium-regulating protein, ryanodine, better known as halothane gene (Maganhini et al. 2007).

Until 24 hours after bleeding, changes occur in muscle causing a degradative and normal pH reduction. However, because of the high sensitivity of animals to stress, there is an excessive glycogen burning in musculature shortly before or shortly after slaughter. This causes higher concentration of lactic acid and rapid reduction of muscle pH, while carcass temperature is still high. Thus, there are actions of proteases that denature muscle proteins, resulting in PSE meat (Barbut et al. 2008).

At low pH, the myofibrilar proteins get near to the isoelectric point, presenting maximum approximation between thick and thin filaments and reducing the space between it. This makes impossible linking water molecules and filaments, reducing the water retention capability. Once water is out of muscle cells and filaments are more attenuated, there is
reflection of incident light, so the flesh become pale. As a consequence, the PSE meat texture is characterized by flaccidity, exudation and pale color (Rosenvold & Andersen 2001).

This type of meat is undesirable for consumers and cannot be marketed in its natural state. Due to low quality, it is intended for manufacture of processed products, which also present problems owing to increased cooking losses, decreased fat emulsification capacity and altered texture of products, resulting in considerable economic losses for industry (O’neill et al. 2003).

There are some factors that can influence the incidence of PSE meat. Besides genetics, among the main causes that may affect meat quality are the extrinsic factors, which involves pre-slaughter handling, since ante-mortem stress by management contribute to the appearance of PSE meat. This extrinsic factors are: long period of fasting, transport from farm to slaughterhouse and rest in lairage, loading and unloading animals and social regrouping, all this are moments when animals are exposed to stressors (Gispert et al. 2000, Maganhini et al. 2007, Barbut et al. 2008, Ludtke et al. 2010).

Based on the importance of this alteration in meat quality, the present study aimed to verify the incidence of PSE carcasses in a pig slaughterhouse in Paraná, Brazil. In addition, it was analyzed if there is influence of the extrinsic factors rest time, total fasting time and at farm, and transport time, on the presence of the alteration and which time intervals of these factors result in a lower occurrence of PSE carcasses.

MATERIALS AND METHODS

A total of 1038 carcasses from 35 randomly selected lots were evaluated during September of 2017 in a pig slaughterhouse located in the region of Campos Gerais, state of Paraná, Brazil, with a slaughtering capacity of 3000 heads per day. Ramps were used to board the animals at farm and disembark at slaughterhouse, and handling boards and rattles were used for loading. Animal density was respected according to the type of truck used for transport. There was a mixing of unfamiliar lots during the board at farm and in the lairages. The animals had free access to water and, on hotter days, were given a sprinkler to reduce thermal stress. Desensitization was made using a carbon dioxide chamber, followed by bleeding.

The pH of 1038 carcasses was verified at the cooling chamber entrance, approximately 45 minutes (pH$_c$) after beginning slaughter, and Longissimus dorsi was the muscle used for insertion of the Testo 206-pH 2® pH meter. Carcasses with pH$_c$$\leq$5.8 were classified as PSE, and those with pH$_c$$>$5.8 as normal (Velazco 2001, Santiago et al. 2012).

In order to evaluate the effects of extrinsic factors, the parameters were divided into classes according to their duration in hours: rest time in pre-slaughter pens (04h01 to 07h; 07h01 to 10h; 10h01 to 13h; 13h01 to 16h; and 16h01 to 19h); total fasting time (11h01 to 14h; 14h01 to 17h; 17h01 to 20h; 20h01 to 23h; 23h01 to 26h; and 26h01 to 29h); fasting time at farm until loading (03h01 to 05h; 05h01 to 07h; 07h01 to 09h; and 09h01 to 11h); and time of transport from farm to the slaughterhouse (01h01 to 02h; 02h01 to 03h; 03h01 to 04h; and 04h01 to 05h).

The total PSE incidence was obtained using the number of PSE positive animals (pH$_c$$\leq$5.8) in relation to the total number of animals evaluated, expressed as a percentage. The influence of each extrinsic factor on the incidence of PSE was measured by the number of animals diagnosed as PSE in relation to the number of heads slaughtered in each time division evaluated, and
the chi-square test ($\chi^2$) was performed using the RStudio software (version 1.1.463) to verify if there is an association between each factor and the PSE frequency, using a significance of 5%.

RESULTS

All animals came from cooperated farms of the slaughterhouse, with an average live weight of 116 kg, non-free genetics of halothane gene, of both sexes, and the males were surgically castrated. Of the lots analyzed, only one had a dead pig during transportation.

Of the 1038 carcasses analyzed, 199 were positive for presence of PSE, representing 19.17%. There was a wide pH variation at 45 minutes after the beginning of slaughter, with a higher frequency in range of 6.0 to 6.3. The frequencies of $\text{pH}_{45}$ are shown in Figure 1.

Resting time, total fasting time, fasting time at farm and transport time had a significant influence ($p < 0.05$) on pH of the carcasses at 45 minutes after slaughter, as well as the incidence of PSE meat.

There was an increase in $\text{pH}_{45}$ with a concomitant reduction in PSE carcass incidence, when rest time was between 04h01 and seven hours (7.07%). Periods higher than seven hours increased PSE incidence, with the highest incidence observed for animals whose rest period ranged from 10h01 to 13 hours (40.00%) (Table I).

For the fasting time from farm to slaughter, there was a lower incidence of PSE carcasses when time was between 14h01 to 17 hours, since only 4.21% carcasses presented PSE meat (Table II).

For the fasting time at farm to the moment of loading, there was a lower incidence of PSE carcasses when time was between 03h01 to five hours, since 5.83% carcasses presented PSE meat (Table III).

And for transport time from farm to slaughterhouse, there was a lower incidence of PSE carcasses when time was between 02h01 to three hours, since 15.71% presented PSE meat. Despite this, there was no significant statistical difference between the transports below four hours, showing that all these periods are feasible. Periods above 04h01 raised the PSE incidence to 31.67% (Table IV).

Figure 1. Histogram of pH frequency at 45 minutes post mortem of 1038 carcasses analyzed in a slaughterhouse in Paraná.
### Table I. Incidence of PSE carcasses as a function of rest time.

| Rest time    | Nº of heads slaughtered | Nº of PSE carcasses | % PSE |
|-------------|-------------------------|---------------------|-------|
| 04h01 to 07h| 311                     | 22<sup>a</sup>      | 7.07  |
| 07h01 to 10h| 210                     | 68<sup>b</sup>      | 32.38 |
| 10h01 to 13h| 30                      | 12<sup>c</sup>      | 40.00 |
| 13h01 to 16h| 308                     | 55<sup>d</sup>      | 17.86 |
| 16h01 to 19h| 179                     | 42<sup>d</sup>      | 23.46 |

Values followed by distinct letters in the 3<sup>rd</sup> column differ significantly by χ<sup>2</sup> test (p <0.05).

### Table II. Incidence of PSE carcasses as a function of total fasting time.

| Total fasting time | Nº of heads slaughtered | Nº of PSE carcasses | % PSE |
|--------------------|-------------------------|---------------------|-------|
| 11h01 to 14h       | 150                     | 16<sup>a</sup>      | 10.67 |
| 14h01 to 17h       | 190                     | 8<sup>b</sup>       | 4.21  |
| 17h01 to 20h       | 150                     | 50<sup>c</sup>      | 33.33 |
| 20h01 to 23h       | 145                     | 19<sup>d</sup>      | 13.30 |
| 23h01 to 26h       | 170                     | 40<sup>d</sup>      | 23.53 |
| 26h01 to 29h       | 233                     | 66<sup>f</sup>      | 28.33 |

Values followed by distinct letters in the 3<sup>rd</sup> column differ significantly by χ<sup>2</sup> test (p <0.05).

### Table III. Incidence of PSE carcasses as a function of fasting time at farm.

| Fasting time at farm | Nº of heads slaughtered | Nº of PSE carcasses | % PSE |
|----------------------|-------------------------|---------------------|-------|
| 03h01 to 05h         | 120                     | 7<sup>a</sup>       | 5.83  |
| 05h01 to 07h         | 515                     | 91<sup>b</sup>      | 17.67 |
| 07h01 to 09h         | 290                     | 68<sup>c</sup>      | 23.45 |
| 09h01 to 11h         | 113                     | 33<sup>d</sup>      | 29.20 |

Values followed by distinct letters in the 3<sup>rd</sup> column differ significantly by χ<sup>2</sup> test (p <0.05).

### Table IV. Incidence of PSE carcasses as a function of transport time.

| Transport time       | Nº of heads slaughtered | Nº of PSE carcasses | % PSE |
|----------------------|-------------------------|---------------------|-------|
| 01h01 to 02h         | 258                     | 43<sup>a</sup>      | 16.67 |
| 02h01 to 03h         | 350                     | 51<sup>a</sup>      | 15.71 |
| 03h01 to 04h         | 250                     | 48<sup>a</sup>      | 17.60 |
| 04h01 to 05h         | 180                     | 57<sup>b</sup>      | 31.67 |

Values followed by distinct letters in the 3<sup>rd</sup> column differ significantly by χ<sup>2</sup> test (p <0.05).
DISCUSSION

The results obtained in this work are similar to literature. In the southern region of Brazil, Maganhini et al. (2007) observed a prevalence of 22.83% and Andrade et al. (1993) found 17.2% of PSE carcasses in Paraná. Faucitano et al. (2010) obtained a result of 21% of PSE meat in Canada. In Mato Grosso do Sul, Brazil, Santiago et al. (2012) found a prevalence of 10.1% and Caldara et al. (2012) obtained 10.06% of PSE pork meat. Culau et al. (1994) identified, in Rio Grande do Sul, Brazil, 30.69% of PSE carcasses. These divergent results can be explained by a large number of factors, but mainly by genetic and pre-slaughter management, since it has a great influence on appearance of this alteration.

The Longissimus dorsi muscle used to evaluate the initial pH (45 minutes post-slaughter) was used by several authors, among them Caldara et al. (2012), Santiago et al. (2012), Ludtke et al. (2010), Faucitano et al. (2010), Maganhini et al. (2007) and Culau et al. (2002). This muscle is one of those with highest glycolytic potential due to transformation of an amount of carbohydrate into lactic acid, therefore it is one of the most chosen for pH evaluation owing to greater probability of developing PSE condition (Murray 1995).

The halothane gene is one of the factors that contribute most to more severe PSE, however, PSE carcass frequency is not only due to the halothane genotype (Culau et al. 2002). Pre-slaughter management is one of the most important stages of production, because animals are exposed to several stressors, resulting in carcasses with quality problems (Ludtke et al. 2010). About management, the time of transportation to slaughterhouse, the ambient temperature during transport, the handling for loading and unloading animals, the incline of the ramp, the pre-slaughtering fasting time and lairage time are very important (Oda et al. 2004).

Adoption of a suitable pre-slaughter rest period is necessary for animals to eliminate excess lactic acid accumulated in muscles due to stress, and to restore their homeostatic balance (Dalla Costa et al. 2006). Higher blood lactate concentration is associated with lower initial pH value, higher meat temperatures and more skin excoriations, denoting pre-slaughter stress (Dokmanovic et al. 2015). Usually, two to three hours of rest are enough to recover from stress of transport without increasing problems of prolonged fasting and skin abrasions (Warriss et al. 1998). For Santiago et al. (2012), the most adequate time for recovering and reduction incidence of PSE meat was between six and eight hours and for Van Der Wal et al. (1997), two to four hours.

However, lairage period depends on the logistics of establishment, and in this study, the shortest rest period began at 04h01 to seven hours, which was the time when there was a lower incidence of PSE meat. Since Brazil has a tropical climate, the stress of animal handling and transportation is much more severe when compared to regions with milder climates (Santiago et al. 2012). Moreover, the hierarchy is established only about two hours and after this the animals start the lairage period (Grandin 1994). The animals in this study were submitted to mixing of unfamiliar batches during transport and resting period, therefore, the increase in PSE carcass incidence after seven hours rest may be related to the increase of aggression between animals submitted to very prolonged waiting periods. Depending on duration of resting time, it may lead to increased skin lesions and appearance of hard, dry and dark (DFD) meat (Gispert et al. 2000).

Swine kept fasted before slaughter contribute to feed economy at farm, and
in addition to reducing mortality rate, prevents vomiting during transport, reduces contamination of carcasses in production line by preventing spread of bacteria through feces, and improves meat quality by not negatively affecting glycogen stores, muscle water loss and color (Dalla Costa 2006). Food restriction, even if it leads to feed economy, also results in increased aggressiveness, especially after mixing animals from different batches (Turgeon & Bergeron 2000). Chevillon (1994) considers a total fasting not more than 24 hours in order to reduce losses of carcass yield. Magras et al. (2000) recommends a total period of 22 to 28 hours to reduce stomach weight. Warriss (1994) suggests a total fasting time of 8 to 18 hours. Periods over 24 hours cause excessive energy expenditure, leading up to 1% of carcass weight loss (Murray 2000), as well as compromising well-being, and reducing meat quality by raising pH at beginning of period post-mortem, as demonstrated by the results of this study. In Brazil, according to Ordinance Nº62, dated May 10, 2018, total fasting, including transportation and rest time, should be at least eight hours (Brasil 2018). According to the present study, from 14h01 to 17 hours of total fasting resulted in lower frequency of PSE carcasses.

There are divergences in relation to recommendations of fasting time at farm. Chevillon (1994) in France and Gispert et al. (2000) in Spain consider a farm fasting from 12 to 18 hours ideal, however Murray (2000) recommend 10 to 24 hours and Eikelenboom et al. (1991) recommends 16 to 24 hours of fasting. Dalla Costa et al. (2010) recommends the adoption of 15 hours of fasting at farm to reduce effects on meat quality. In Canada, at least five hours of fasting are recommended (NFAAC 2014). There is a great influence of logistic between farms and abattoirs in the recommendation of fasting time. In Brazil, at discretion of the Federal Inspection Service, animals that have begun fasting at farm may be slaughtered, provided that they have rested for at least two hours at abattoir (Brasil 2018). The fasting time at farm from 03h01 to five hours resulted in a lower frequency of PSE carcasses, and all the animals remained at rest in the establishment for at least 04h01. Fasting times at farm from 05h01 have considerably increased the incidence of PSE carcasses, diverging from literature.

Although a 12 to 18 hour fasting at farm decreased mortality rate in Spanish slaughterhouses (Guàrdia et al. 1996), there were no significant deaths at any time intervals in this study. The only batch with a dead pig was in the recommendations found for total and farm fasting time and rest time, but it was outside the recommended transport time (04h01 to five hours).

The transport is influenced mainly by animal density, time of transport and distance traveled (Pèrez et al. 2002). Time is more important than distance, since factors that affect time like gathering animals at farms and characteristics of roads, can represent more pronounced effects in animal stress. In addition, when very long, it contributes to raise pH meat and cause fatigue to animal (Silveira 2006). The number of animals in each truck partition used to transport to the slaughterhouse of this study was calculated according to each type of truck and the average live weight of the pigs so that density was adequate. The recommendation is that transport time should be minimum and at most three hours (Warriss 1996). However, journeys of less than one hour may be more harmful than longer ones, because pigs should have time to recover from stress of loading (Bradshaw et al. 1996). The results found are similar to literature, since transport time between one and four hours did not present statistical difference, and more than
04h01 significantly increased the incidence of PSE.

The economic losses of PSE meat to industry are related to its use in preparation of meat products. Due to impairment of meat proteins functional properties, it can be used up to a certain limit for production of some fermented products and certain types of emulsions and is unsuitable for production of cooked ham and other cooked cured products (Wirth 1986). Caldara et al. (2012) calculated an annual loss of approximately R$340,800,00 in an industry in Mato Grosso do Sul with 10.06% of pig meat affected by PSE condition. Owen et al. (2000) showed that some researchers estimated economic losses from PSE meat, estimating that the United States loses US$ 32 million annually and Australia and the United Kingdom lose about US$20 million, due to appearance of this type of meat.

CONCLUSION

The incidence of pork PSE meat was 19.17%, and this was influenced by rest time of the animals in pre-slaughter pens, total fasting time, fasting time at farm until slaughter and time of transport of the animals from farm to abattoir. The hours that presented lower frequency of this problem were from 04h01 to seven hours, from 14h01 to 17h00, from 03h01 to five hours and from 02h01 to three hours, respectively.

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