



The effect of stress during lairage and stunning on muscle metabolism and drip loss in Danish pork

by

Susanne Støier^{1*}, Margit Dall Aaslyng¹, Eli V. Olsen¹, Poul Henckel²

1. Danish Meat Research Institute, Maglegårdsvej 2, DK-4000 Roskilde, Denmark
2. Danish Institute of Agricultural Sciences, Dept. of Product Quality, Research Center Foulum, Box 50, DK-8830 Tjele, Denmark

*To whom correspondence should be addressed: email sst@dmri.dk,
Telephone +45 46303030, telefax +45 46303132

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Abstract

The effect on meat quality of a low stress handling system (LSS) compared to a traditional handling system (TS) was investigated on Duroc x (Landrace x Yorkshire) (n=117) and (Hampshire x Duroc) x (Landrace x Yorkshire) pigs (n=110) under commercial conditions. In the low stress handling system the pigs were kept in groups of 15 during lairage and movement up to the stunner. Before the stunner the groups were divided into three groups of five pigs for the CO₂-stunning in a specially designed set-up. The pH and temperature were determined in *m. longissimus dorsi* (LD) and *m. biceps femoris* (BF) at various times post mortem. Immediately after exsanguination a biopsy was taken from the LD and analysed for the concentration of glycogen, lactate and creatine phosphate. The day after slaughter the pH was determined in the LD, BF, *m. semimembranosus* (SM) and *m. semispinalis capitis* (SC). The temperature was determined in the LD and BF, the internal reflectance was determined in the LD, SM and BF, the colour was determined in LD, the drip loss was determined in LD and BF and the amount of blood splashing/bruising was evaluated in LD. There was a tendency to a higher concentration of creatine phosphate in the LSS-group (p=0.06). The pH in both the LD and BF on the day of slaughter decreased more slowly from 5 min post mortem to 40 min post mortem in the LSS-group than in the TS-group (p<0.001). From 40 min to 6 h post mortem the rate of the pH decline was similar in the two groups producing the lowest pH-level in the TS group. The day after slaughter the pH was similar in the two groups in the LD and SC whereas in the BF and SM it was lower in the LSS-group than in the TS-group. The drip loss was lower in the LSS-group in both LD (p<0.01) and BF (p<0.05) whereas the internal reflectance was only different in LD with the lowest value in the LSS-group (p<0.001). The lightness (L*) was higher in the LSS-group (p<0.05). There was no effect of stunning system on the amount of blood splashing/bruising in the LD. The study showed that by using a low stress stunning system it is possible to decrease drip loss, possibly by increasing the concentration of creatine phosphate and thereby delaying the acceleration of pH fall in muscles after death.

Keywords

Pigs; Low stress handling; Drip loss; pH; Internal reflectance; Creatine Phosphate, Lactate

1. Introduction

The water holding capacity (WHC) of pork is of great financial importance. Previously the genetic defect that causes Malignant Hyperthermia, also called "the halothane gene", was a major problem by inducing PSE meat which was pale, soft and with very high drip loss. PSE was due to a fast pH decline which induced pronounced protein denaturation. Today the frequency of the halothane gene is very low in Denmark - less than 2 % carriers (Nn) and no homozygotes (nn) (Ulla Gam Hansen, pers. comm., 1999). Still a high drip loss does some times occur but without the pale colour. This condition is termed RSE meat (red soft exudative) (Kauffman, Sybesma, Smulders, Eikelenboom, Engel, van Laack, Hoving-Bolink, Sterrenburg, Nordheim, Walstra, van der Wal, 1993) and, unlike PSE meat, is not a result of a specific genetic condition.

Stress immediately before stunning has been shown to decrease the early post mortem pH, and increase the temperature and the concentration of lactate up to 70 min post mortem (Wal, Engel & Hulsegge, 1997; Brown, Warriss, Nute, Edwards & Knowles, 1998; D'Souza, Dunshea, Warner & Leury, 1998a; D'Souza, Warner, Dunshea & Leury, 1998b; Wal, Engel & Reimert, 1999). The WHC seems also to be decreased by stress immediately before stunning without development of PSE meat (D'Souza et al., 1998b; Wal et al., 1999). This indicates that the metabolism early post mortem is of importance for the development of drip loss even in halothane gene-free pigs.

In many traditional abattoir systems unfamiliar pigs are handled together in large pens during lairage with a high incidence of fighting (Barton Gade, Blaabjerg & Christensen, 1992).

Movement from the lairage pens to the stunner is often in large groups until the race where electric goads are necessary to force the pigs one by one into the stunner. This is against the nature of the pig and is therefore a stressful treatment in the minutes up to stunning. To decrease this stress a new low stress system (LSS) has been developed. In this system the pigs are kept in groups of 15 during lairage in a small pen system. This small pen system reduces aggression

during lairage even though some pigs may be unfamiliar with one another. Pigs therefore lie down to rest more quickly than when larger groups are used. The same groups are maintained during movement up to the stunner. They are allowed to walk at their own pace and no electric goads are used. Before the stunner the groups are divided into three groups of five pigs for the CO₂-stunning in a specially designed set-up (Barton Gade et al., 1992; Barton Gade, Blaabjerg & Christensen 1995; Christensen & Barton-Gade, 1997). The systems have been especially adapted to pig behaviour rather than the reverse. The system facilitates forward movement of the pigs, reduces interaction with humans and allows driving to the stunning point without the use of electric goads. It can therefore be regarded as a low stress treatment both during lairage and in the last minutes prior to stunning.

The objective of this study was to investigate if this newly developed low stress handling system would improve the meat quality, especially water holding capacity, in comparison with a traditional stunning (TS) system under commercial conditions.

2. Materials and methods

2.1. Instruments and analysis

The pH was determined with a Knick Portamess pH-meter no. 751 (Berlin, Germany) with an Ingold LOT glass electrode type 3120 (Mettler Toledo, Urdorf, Switzerland)). The temperature was determined with a Testotherm thermometer with a Ni-Cr-Ni probe type 06000-2694 (Buhl & Bundsoe, Hedehusene, Denmark). For determination of internal reflectance an MQM-instrument (SFK-Technology Soborg, Denmark) was used (Borggaard et al., 1989). The pH, temperature and internal reflectance were all determined between the 4th and 5th lumbar vertebrae in the *m. longissimus dorsi* (LD), and in the centre of the *m. biceps femoris* (BF), *m. semimembranosus* (SM), and *m. semispinalis capitis* (SC). The colour was determined one hour after excision with a

Minolta CR300 (Minolta, Japan) as an average of 8 sampling sites on a 2.5 cm thick slice of LD taken at the 7th lumbar vertebra. The drip loss was determined using the container method described by Rasmussen & Andersson (1996). The amount of blood splashing/bruising was evaluated on the silverside and the bone side of the LD, on a scale from 1 (no blood splashing) to 4 (blood splashing leading to a serious deterioration in quality).

The concentration of glycogen and lactate were determined spectrophotometrically as described by Passonneau & Lowry (1993). Creatine phosphate was analysed by HPLC as described by Henckel, Karlsson, Jensen, Oksbjerg & Petersen (2000).

2.2. Experiment

Pigs from four producers - two producing crosses between Duroc (boar) and Landrace-Yorkshire (sow) breeds (DLY) (n=117) and two mainly producing crosses between Hampshire-Duroc (boar) and Landrace-Yorkshire (sow) breeds (HDLY) (n=110) - were delivered to a commercial slaughterhouse two days per week for two weeks. The pigs were assumed to be halothane negative (NN) even though this was not determined, as the gene has been practically eliminated from the breeds in Denmark (Ulla Gam Hansen, pers. comm., 1999). On each day both DLY and HDLY crosses were slaughtered. The pigs were kept pen-wise on the vehicles. On arrival at the abattoir the pigs from each producer were divided in two groups of approximately 15 per group. One group from each producer was given a special low stress treatment. In the low stress treatment the pigs were kept in the groups of 15 during lairage for two hours and while moving to the stunner. The pigs were allowed to walk at their own pace and no electric goads were used. Just before the stunner the groups were divided into subgroups of 5 pigs that were stunned together. The CO₂-concentration was 84% in the first position and 90 % at the bottom of the pit. Through-time was 150 seconds; maximum stun-stick interval was 75 seconds. The slaughter rate was 360 pigs per hour. The other group was given a traditional treatment. They were mixed in lairage with pigs from the other farms in groups of 30 pigs. The lairage time was two h. They were stunned

individually in a traditional CO₂ stunner after an 8 m race where the use of electric goads was necessary to drive the pigs forward. The CO₂ concentration was at least 65% in the second position and 84% at the bottom of the pit. The concentration in the first position was not possible to determine. The two groups were slaughtered with a 15 min interval.

After exsanguination, about 5 min post mortem, the pH and temperature were determined in the LD. In the first week a biopsy of 100-200 mg was taken in the centre of the LD around the curvature of the last rib from 48 pigs from the low stress treatment and 29 pigs from the traditional treatment with similar numbers of DLY and HDLY in both groups. After 40 min and 6 h the pH and temperature were determined in the LD and BF. Between 22 and 24 h post mortem the pH was determined in the LD, BF, SM and SC and the temperature was determined in the LD and BF. The internal reflectance was determined in the LD, SM and BF, the colour was determined in the LD, drip loss was determined in the LD and BF, and the amount of blood splashing/bruising was evaluated in the LD.

2.3. Statistical analysis

Data from the measurements of meat quality and metabolite concentrations were analysed by an analysis of variance (SAS Institute Inc., N.C., USA) using the following model:

$$Y_{ijkl} = \gamma + \text{treatment}_i + \text{producer}_j + \text{slaughter day}_k + (\text{slaughter day} * \text{producer})_{jk} + (\text{treatment} * \text{producer})_{ij} + (\text{slaughter day} * \text{treatment})_{ik} + \varepsilon_{ijkl}$$

In an initial analysis it was found that no three factor interaction was present. The carcass weight and the meat percentage can also influence the meat quality, but they were not included in the model as they were found to be similar in the two experimental groups by an analysis of variance.

The amount of blood splashing/bruising produced by the two stunning methods was compared by a χ^2 -analysis.

3. Results

The decrease in pH on the day of slaughter can be seen in Figure 1. The pH in the LD was significantly higher ($p < 0.01$) in the low stress stunning group (LSS-group) 5 min post mortem compared to the traditional stunning group (TS-group). Furthermore the rate of pH fall was lower in the LSS-group up to 40 min post mortem ($p < 0.01$). The higher pH was maintained until 6 h post mortem, but the rate of the pH decrease was similar in the two stunning groups between 40 min and 6 h. The day after slaughter the pH in the LD was similar in the two groups. In the BF the pH was determined for the first time at 40 min post mortem. At this time the pH was significantly higher ($p < 0.01$) in the LSS-group. As in the LD the rate of the pH fall was similar for the two groups between 40 min and 6 h but, due to the higher pH at 40 min, the pH at 6 h was higher in the LSS-group ($p < 0.01$). The day after slaughter this was changed and the pH was lower in the

LSS-group ($p < 0.001$). In the SM the pH the day after slaughter was likewise lower in the LSS-group (pH 5.57 compared to pH 6.08, $p < 0.01$) whereas no difference was seen in the pH of the SC (pH 6.06). The effect of stunning method was independent of crossbreed combination, as there were no interactions between producer and stunning treatment for any of the pH-values.

There was no significant difference in temperature between the two groups in the LD but in the BF the temperature was about 1°C lower in the LSS-group 40 min post mortem ($p < 0.001$) (data not shown). However, there was a significant effect of both producer and slaughter day, and a significant interaction between these, and this might have masked a small difference in temperature between the two stunning treatments.

The concentration of the metabolites in the LD shortly after exsanguination is shown in Table 1. Due to practical problems in taking the biopsies only 29 samples were taken from the traditional stunning group (TS-group) compared to 48 from the LSS-group. There was a tendency for these to be a higher concentration of creatine phosphate in the LSS-group compared to the TS-group ($p = 0.06$) and there was a significant difference between the producers ($p < 0.05$). The concentration of glycogen was not affected by either stunning treatment, producer, or crossbreed combination, but the variation was significantly higher in the pigs from one of the HDLY-producers compared to the other three producers. There was no significant effect of stunning treatment on the concentration of lactate, but there was an effect of producer. This effect was independent of crossbreed combination.

The meat quality the day after slaughter is shown in Table 2. Drip loss was significantly lower in the LSS-group in LD ($p < 0.01$) and BF ($p < 0.05$). There was no interaction between stunning method and either producer or slaughter day and the effect of the stunning method was therefore independent of crossbreed combination and day to day variations.

The internal reflectance reflects the degree of protein denaturation and thereby indicates the water holding capacity of the meat (Borggaard et al., 1989). As can be seen in Table 2 the internal reflectance was significantly lower in the LSS-group in LD ($p < 0.001$). No difference was seen in BF (Table 2) or SM (data not shown).

The colour was determined in LD. The lightness expressed by L^* -value was higher in the LSS-group ($p < 0.05$) (Table 2). For a^* -value and b^* -value a significant interaction between producer and stunning method was seen (a^* -value: $p = 0.01$, b^* -value: $p = 0.02$). For one producer the a^* and b^* values were lower in the LSS-group whereas no difference was seen between stunning methods for the other three producers.

There were no differences between the two groups in the amount of blood splashing/bruising between the two stunning systems. In both systems at most only 5 % of carcasses were given a score indicative of a level that requires trimming.

4. Discussion

In a traditional lairage and stunning system the pigs are handled in a way that is against their nature. The pigs are often mixed with unfamiliar pigs during transportation and lairage, which induces fighting (Barton Gade et al., 1992). This is combined with movement in big groups up to the race and individually the last metres prior to stunning. In most studies of the effect of stress on meat quality the focus has been on the handling in a specific period of the time up to stunning like comparison of a stressful handling and a gentle handling during transportation and lairage (Lundstrøm et al., 1987; Warriss et al., 1992), or during movement up to the stunner and the last minutes before stunning (D'Souza et al 1998a, D'Souza et al 1998b, Wal et al. 1999). In addition most of these studies have been performed at research facilities. In this investigation we have

focused on a gentle handling of the pigs the whole way from the producer, during transportation, lairage, movement to the stunner and into the CO₂-stunner. The experiment was carried out under commercial conditions at a commercial slaughtering rate, which ensures that the results are of commercial relevance.

Stressful handling of pigs the last minutes before stunning is known to increase the concentration of the stress hormone adrenaline in the blood (Troeger & Woltersdorf, 1989). Adrenaline increases the rate of glycolysis and an increase in the concentration of lactate is therefore expected. D'Souza et al. (1998a) showed that a negative handling just before stunning actually did increase the concentration of lactate and decrease the concentration of glycogen. In our experiment we found that the pH 5 min post mortem was lower in the TS-group compared to the LSS-group. This indicates that a difference in lactate concentration was present even though it was not detected in the biopsies. The rate of pH fall was higher in TS-group compared to the LSS-group from 5 to 40 min, but from 40 min to 6 h the rate of fall was similar in the two groups even though the absolute values of the pH were still different (Figure 1). This could be due to a higher concentration of creatine phosphate after exsanguination (Table 1). By giving the pigs the low stress handling treatment the need for energy before stunning was lower and the concentration of creatine phosphate therefore higher after exsanguination. As the anaerobic degradation of glucose to lactate is initiated when the concentration of creatine phosphate is low (Pearson & Young, 1989) this could explain why the acceleration of pH-fall was delayed. The rate of pH decrease once started (from 40 min to 6 h) was independent of stunning procedure.

A significantly higher drip loss was seen in the TS-group compared to the LSS-group in both the LD and BF. In the LD the TS resulted in a higher internal reflectance compared to the LSS, but it was not high enough for the meat to be described as PSE in any of the pigs. The negligible difference in the lightness (L^*) between the stunning groups supports the view that the variation in drip loss between the groups was not due to PSE. A low stress treatment prior to stunning has

therefore reduced the drip loss in normal meat. This is in agreement with van der Wal et al. (1999) who found in a research abattoir that a standardised stress in the last minutes before stunning decreased the water holding capacity of the meat. They also found that a high degree of coercion of the pig did result in a lower water holding capacity.

It has been shown that the effect of pre-slaughter handling on meat quality shows interactions with sex (gilts and boars) (Moss & Robb, 1978). In this study the only differences between the sexes (gilts and castrates) were in temperature in the LD 5 min post mortem and in pH 6 h post mortem whereas the meat quality the day after slaughter was similar between the sexes. Whether there were any interactions between crossbreed combinations was investigated. The level of drip loss and $pH_{ultimate}$ were different between the two crossbreed combinations but the effect of the stunning treatment did not interact with either crossbreed combination or day to day variations. The decrease in drip loss produced by a low stress handling treatment compared to traditional handling must therefore be expected to be consistent from day to day.

It can be concluded that using this special low stress stunning system even under commercial conditions did delay the acceleration of the pH-fall early post mortem possibly because the concentration of creatine phosphate after exsanguination was higher compared to the traditional stunning system. The day after slaughter drip loss was correspondingly lower in the low stress stunning system without any real PSE developing. This shows that even in normal meat the amount of drip loss can be decreased by reducing stress prior to stunning.

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Legends to figures

Figure 1 pH in longissimus dorsi and biceps femoris on the day of slaughter

Table 1

Creatinephosphate, glycogen, and lactate concentrations in biopsies from the LD taken shortly after exsanguination after a low stress handling procedure (n=34) compared with a traditional handling procedure (n=28)

	Low stress stunning	Traditional stunning	P(difference)
Creatinephosphate ($\mu\text{mol/g}$)	7.1 (0.7) ^a	5.2 (0.7)	P=0.06
Glycogen ($\mu\text{mol/g}$)	57 (4.3)	53 (4.3)	
Lactate ($\mu\text{mol/g}$)	30 (1.7)	33 (1.8)	P<0.05

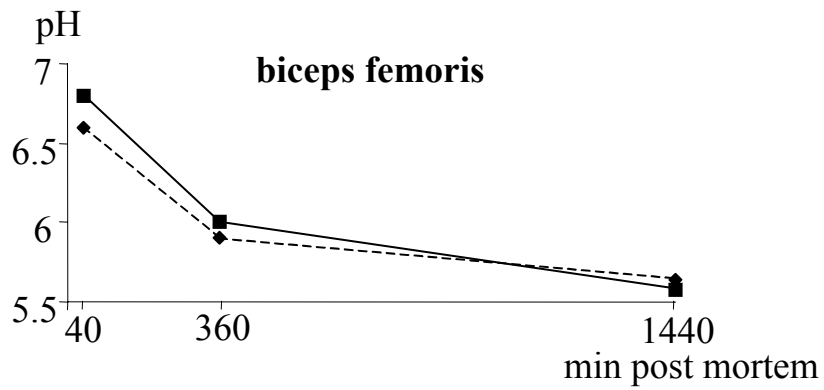
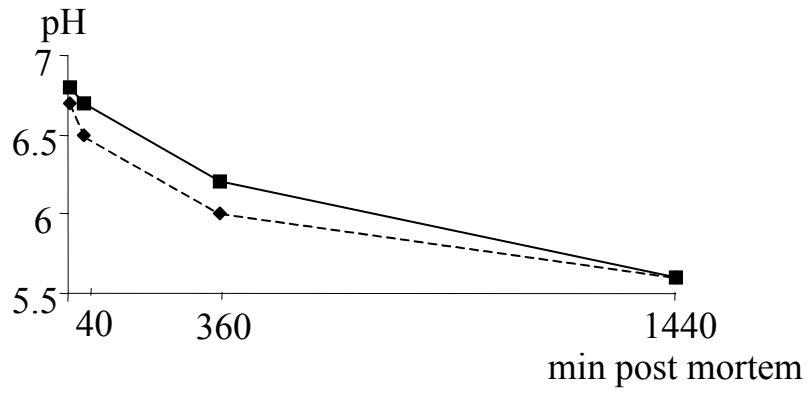
^a least squares means (se)

Table 2

Meat quality of pork after a low stress handling procedure (n=110) compared with a traditional handling procedure (n=117)

	longissimus dorsi			biceps femoris		
	Low stress	Traditional	P(difference)	Low stress	Traditional	P(difference)
Drip loss, %	3.2 (0.1) ^a	3.7 (0.1)	P<0.01	1.6 (0.1)	1.9 (0.1)	P<0.05
Internal reflectance	43 (0.7)	47 (0.7)	P<0.001	74 (0.8)	75 (0.8)	
L*	51 (0.3)	50 (0.3)	P<0.05	not determined	not determined	

^a least squares means (se)



—■— low stress stunning ---◆--- traditional stunning