 DOI: 10.24099/vet.arhiv.2542

The effect of stunning methods on stress and meat quality parameters in pigs

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STAJKOVIĆ, S., D. VASILEV, M. DIMITRIJEVIĆ, N. ČOBANOVIĆ, N. ARABASIL: The effect of stunning methods on stress and meat quality parameters in pigs. Vet. arhiv 94, 297-304, 2024.

ABSTRACT

A total of 100 commercial market pigs were randomly allocated to two stunning treatments: carbon dioxide (CO_2) and electrical stunning, at a commercial abattoir. The effects of the two stunning methods on pig stress and meat quality were measured. Blood lactate and cortisol, and postmortem pH (pH45 min; pH24 h), temperature (T45 min), drip loss and sensory color for the *longissimus dorsi, pars lumbalis,* were determined. After electrical stunning, pH45 min and pH24 h was lower than after CO_2 stunning ($P<0.05$), and T45 min was higher ($P<0.01$). There was no effect ($P>0.05$) of stunning on drip loss 24 h and 48 h post-mortem, or sensory color, or on stress parameters, lactate and cortisol. However, individual animal variations of stress markers within the slaughter batch were very high, and their average concentrations indicated high levels of stress. From the perspective of animal welfare and protection, both stunning methods should be conducted properly, according to the defined procedures to minimize stress and to assure adequate meat quality.

Key words: pigs; gas stunning; electrical stunning; meat quality; stress

Introduction

Before slaughter pigs are stunned in order to protect animal welfare and to avoid unnecessary suffering, fear, pain and stress during this process. Stunning is a statutory requirement in the EU (EC, 2009) and in most developing countries. In Europe, one of the most common methods for stunning pigs at slaughter is with CO_2. However, electrical stunning is also widely used (SINDHØJ et al., 2021).

Various studies have compared the two techniques, relative to animal welfare and to meat quality (TERLOUW et al., 2021). For gas stunning, in EU countries the pigs are introduced into a gondola that is lowered into a 7 to 8 m deep pit containing at least 80% of CO_2 at the bottom of the pit (EC, 2009), to induce unconsciousness. The depth of unconsciousness is thought to depend on various factors such as CO_2 concentration,

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ISSN 0372-5480
Printed in Croatia

exposure time and the animal (ATKINSON et al., 2012). The main animal welfare advantage of gas stunning compared to electrical stunning is that pigs can be handled and stunned in groups rather than individually, which has been shown to greatly reduce separation anxiety and distress in pigs (ATKINSON et al., 2012). Also, CO_2 stunning minimizes the fear and stress caused by close human contact, and may diminish the incidence of petechiae and hematomas (ATKINSON et al., 2020). Prior to electrical stunning using a manual tong, it is often necessary to handle the pig individually and restrain it, which causes stress. Automatic systems (mostly used in commercial settings) are equipped with a restrainer, which is also stressful for the animals (TERLOUW et al., 2021). If stunning is performed correctly, the pigs should collapse immediately, followed by a tonic and then clonic seizure.

Inadequate pre-slaughter handling procedures often elevate the blood levels of catecholamines and other stress hormones, including cortisol, as well as lactate (HAMBRECHT et al., 2004). Pre-slaughter stress factors can lead to the rapid depletion of glycogen reserves in the muscles, excessive accumulation of lactic acid, and a decrease in pH values. Lower pH at early post-mortem may result in greater drip loss, and a lighter color of the meat (TERLOUW et al., 2021). Several authors have found no conclusive evidence of the impact of the stunning method (electrical vs. CO_2) on meat quality traits, color and drip loss (TERLOUW et al., 2021).

Overall, the stunning method seems to be important in the management of stress in pigs and in the resultant pork meat quality, but these interactions are likely very complex. Therefore, the objective of this study was to monitor the implementation of two different stunning procedures (electrical and CO_2) and to evaluate their effects on selected stress indicators and meat quality parameters.

Materials and methods

Animals, stunning. Commercial market pigs, [Yorkshire × Landrace] sows, sired with Pietrain boars (n=100), six months of age and with live weights between 92 and 138 kg, were studied in the period from December to May. In that period, the procedure was monitored on six days of sampling (daily with 15-18 animals), as the groups of animals were identified as groups I, II, III, IV, V and VI. The pre-slaughter procedure, slaughtering, processing and cooling were carried out in two slaughterhouses (slaughterhouse A and slaughterhouse B). Groups I, II, III, IV and VI of pigs were transported to slaughterhouse A, and group V was transported to slaughterhouse B. Groups I, III, IV and VI were stunned with CO_2 and groups II and V were electrically stunned.

Pre-slaughter and slaughter. Pigs transported on the same day originated from the same farm. A conventional double-decker pig transport vehicle, supplied with tail-lifts that accommodates a maximum of 13 animals, was used for groups I, II, III, IV, and VI, and a non-specialized vehicle, for group V. According to the duration of transport, the animals underwent short transport, up to 4 h (groups III, IV, V and VI) and long transport, over 4 h (groups I and II). During loading and unloading, no sticks or electric prods were used to move the pigs. The duration of lairage depended on the dynamics of slaughter and the organization of work in the slaughterhouse, and lasted up to 3 h (group V) or over 3 h (group I, II, III, IV and VI). During lairage, the pigs were not mixed.

The CO_2 dip lift stunner (Butina APS, Denmark) was set at 90% CO_2 and the pigs were exposed to CO_2 for between 20 s and 33 s, in groups of 3 to 4 animals. The stun to stick interval was from 20 s to 103 s (group I from 20 s to 45 s, group III from 40 s to 76 s, group IV from 35 s to 65 s, group VI from 30 s to 103 s).

Pigs from group II were head-only electrically stunned at slaughterhouse A (3 to 30 s), and pigs from group V were head-only electrically stunned at slaughterhouse B (10 s) with a minimum current of 1.3 A, in batches of 3 to 5 animals without restraints, in an area of 12 m^2. The stun to stick interval for II group was from 35 s to 100 s, and for group V from 30 s to 97 s. Before stunning, the animals were showered with water.

The animals were shackled and hoisted before exsanguination. Following bleeding, the carcasses were processed using conventional industry practices.

Blood sampling and determination of blood lactate and cortisol content. At slaughter, blood samples were taken from each animal, and the lactate content was determined on the spot using a portable lactate analyzer (Lactate Scout, EKF Diagnostic, Magdeburg, Germany), calibrated with a standard solution to ensure accuracy. For determination of cortisol concentrations, blood samples were collected into Vacutainer tubes containing heparin (BD Vacutainer® Heparin Tubes), and were placed on ice (+4°C), and then the Vacutainer tubes were centrifuged within 1 to 2h at 3000 rpm for 10 min to separate plasma. Plasma was transferred into microtubes and stored at -20°C until determination of cortisol concentrations by electrochemiluminescence immunoassay (ECLIA, Elecsys E170, Roche Diagnostics, Switzerland, Belgrade Laboratory, Belgrade, Serbia).

Meat and carcass quality analyses. The carcasses were clearly labeled. Meat quality measurements were carried out 45 min, 24 and 48 h after slaughter on the muscle *longissimus dorsi* (LD), *pars lumbalis*. pH and temperature values were measured using a Testo 205 (Germany) pH-meter, calibrated with pH 4.00 and 7.00 phosphate buffer (Reagecon), at 45 min (pH45 min, T45 min) and 24 h (pH24 h) post-mortem. Meat samples were taken after slaughter for determination of drip loss (100±0.0001 g), and were weighed and stored in a container for 24 h and 48 h at 4°C (HONIKEL, 1998). After storage, the meat samples were reweighed and the percentage of drip loss was calculated. Meat samples for determination of sensory color (2.5 cm thick loin chops) were taken 24 h after slaughter from the LD, and an analytical panel of three trained members assessed the sensory color of meat samples using a 1–6 scaling method (NPPC, 2000), after approximately 60 min of blooming time.

Stun effectiveness rating. When evaluating the effectiveness of CO_2 stunning for each animal, the following parameters were monitored: CO_2 concentration, the number of animals per group, duration of stunning, and the stun to stick interval (GRANDIN, 2010; Welfare Quality® consortium, 2009). When assessing the effectiveness of electrical stunning for each animal, the following parameters were monitored: the strength of the current applied, the regularity of electrode application, the success of stunning at the first attempt, the stun to stick interval (GRANDIN, 2010; Welfare Quality® consortium, 2009). Three observers assessed stunning efficiency for each pig.

Statistical analysis. Statistical analysis of the results was conducted using the software GraphPad Prism version 7.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). All parameters were described by descriptive statistics (mean, standard deviation, minimum and maximum values). The student's t-test was used to examine the effects of the stunning method (CO_2 vs. electrical) on stress and meat quality parameters in the pigs. Values of $P<0.05$ and $P<0.01$ were considered significant.

Results

Characterization of the experimental population. Table 1 shows the mean values of stress and meat quality parameters in the pigs. Blood lactate at exsanguination ranged from 2.10 to 24.10 mmol/L. Plasma cortisol content varied from 82.50 to 1150.0 nmol/L. Values of pH after 45 min ranged from 5.63 to 7.24, and after 24 h from 5.33 to 6.65. The highest carcass temperature was 40.9°C, and the highest drip loss of meat samples after 24 h was 5.67% and after 48 h 8.50%. Sensory color scores ranged from 1.00 to 4.33.

Stunning method. Table 2 shows stress and meat quality parameters in relation to the stunning method. Average blood lactate at exsanguination after CO_2 stunning was 11.92±5.92 and ranged from 2.10 to 24.10 mmol/L, while average blood lactate at exsanguination after electrical stunning was 12.31±5.50 and ranged from 3.80 to 24.40 mmol/L. The average plasma cortisol level after CO_2 stunning was 355.1±140.5 mmol/L and ranged from 82.50 to 774.3 mmol/L, while the average plasma cortisol level at exsanguination after electrical stunning was 420.3±248.6 nmol/L and ranged from 82.53 to 1150.00 nmol/L. Electrical vs. CO_2 stunning was related to lower meat quality: lower pH45 min (6.37±0.28 vs 6.52±0.31, respectively), higher T45 min (39.72±0.67 vs 38.73±1.45, respectively), and lower pH24 h (5.55±0.16 vs 5.73±0.31, respectively). The stunning method (CO_2 vs. electrical stunning) did not affect the drip loss of the meat samples (P>0.05) after 24 h (2.06±1.05% vs 1.87%, respectively) or after 48 h (3.07±1.67% vs 2.76±1.09%, respectively), or sensory color scores (2.56±0.91 vs 2.72±0.70, respectively).

Table 1. Characterization of the experimental population (n = 100): Stress and meat quality Parameters

Parameter	$\bar{x}$± SD	Min.	Max.
Blood lactate (mmol/L)	12.05±5.77	2.10	24.40
Plasma cortisol (nmol/L)	366±156.2	82.50	1150.0
pH45 min	6.47±0.31	5.63	7.24
pH24 h	5.68±0.28	5.33	6.65
T45 (°C)	39.06±1.33	34.70	40.90
Drip loss24 h (%)	2.00±0.93	0.43	5.67
Drip loss48 h (%)	2.97±1.51	0.66	8.50
Sensory color	2,61±0.85	1.00	4.33

pH45 min and pH24 h - pH values measured 45 min and 24 h post-mortem; T45 min - meat temperature measured 45 min post-mortem; Drip loss24 h and Drip loss48 h – Drip loss measured 24 h and 48 h *post-mortem*

Table 2. Stress and meat quality parameters in relation to the stunning method

Parameter	Stunning with CO2 (n=67)			Electrical stunning (n=33)		
	$\bar{x}$± SD	Min.	Max.	$\bar{x}$± SD	Min.	Max.
Blood lactate (mmol/L)	11.92±5.92^{a}	2.10	24.10	12.31±5.50^{a}	3.80	24.40
Plasma cortisol (nmol/L)	355.1±140.5^{a}	82.50	774.3	420.3±248.6^{a}	82.53	1150.00
pH45 min	6.52±0.31^{a}	5.63	7.24	6.37±0.28^{b}	5.83	6.89
pH24 h	5.73±0.31^{a}	5.33	6.65	5.55±0.16^{b}	5.43	6.11
T45 min (°C)	38.73±1.45^{x}	34.70	40.70	39.72±0.67^{y}	38.10	40.90
Drip loss24 h (%)	2.06±1.05^{a}	0.43	5.67	1.87±0.61^{a}	0.79	3.46
Drip loss48 h (%)	3.07±1.67^{a}	0.66	8.50	2.76±1.09^{a}	0.90	5.62
Sensory color	2.56±0.91^{a}	1.00	4.33	2.72±0.70^{a}	1.50	4.00

pH45 min and pH24 h - pH values measured 45 min and 24 h post-mortem; T45 min - meat temperature measured 45 min *post-mortem*; Drip loss24 h and Drip loss48 h – Drip loss measured 24 h and 48 h post-mortem; different letters within a row indicate a significant difference between groups (a, b - P<0.05; x, y - P<0.01).

Discussion

Stunning procedure. During electrical stunning, it is crucial that the electrodes are positioned correctly in order to achieve immediate unconsciousness. In our study, this was not the case, so 17.7% of the pigs were stunned incorrectly at the first attempt. Stunning in a deep lift system with 90% CO_2 for at least 100 s can be regarded as acceptable in terms of animal welfare, in combination with a stun-to-stick interval of 25 to 35 s (NOWAK et al., 2007). These factors are managed by individual abattoirs according to their own discretion, and according to the technical design of the slaughter line. In our study, the number of pigs per group stunned, the time taken to reach maximum CO_2 concentrations, the total exposure time, and the stun to stick interval, varied and were not adequate. Exposure to the 90% CO_2 atmosphere did not exceed 33 s in all tested groups, and considering that 3-4 pigs were forced into the stunning box in the deep lift, the time needed to exsanguinate the last pig in the group increased (from 20 to 103 s).

Stress parameters. Determining the concentration of cortisol has been the "gold standard" in assessing stress in animals for many years. Data in the literature on cortisol concentrations at exsanguination vary greatly (MORMÈDE, 2007), as was the case in our study (Table 1). The large variation in cortisol levels that we observed could be, at least partly, a consequence of individual variability (susceptibility to stress). MORMÈDE and DANTZER (1978) examined basal cortisol levels in pigs, and found values ranging from 55.18 nmol/L to 165.14 nmol/L. ŚMIECIŃSKA et al. (2011) found even lower cortisol concentrations before transport and stunning (from 55.49 nmol/L to 79.74 nmol/L), indicating that the animals in our study were stressed (Table 1). Cortisol levels can be affected by breed, gender, age, food deprivation, individual variability, repeated noise, time of day, handling and transportation conditions, lairage time, stunning treatment, and other factors (MORMÈDE, 2007). In the current study, however, age, food deprivation, genetic background and time of day did not affect cortisol levels greatly, because the pigs were of the same cross-breed and age, and were all slaughtered in the morning to limit the effect of diurnal cortisol fluctuation. In the current study, there was no significant difference between cortisol concentrations in pigs stunned with CO_2 and those electrically stunned (Table 2). Despite notable differences in the average levels of cortisol (Table 2), the lack of significance, to our opinion, is mainly due to high variations within the batches. Lactate and body temperature are quick responders to handling-induced stress and physical activity in swine (EDWARDS et al., 2010). Plasma lactate increases to its maximum level within four minutes of physical exercise, and returns to basal level in 2 h (CORREA et al., 2013). WARRISS et al. (1994) also emphasized the sensitivity and responsiveness of lactate to pre-slaughter stress. The average blood lactate at exsanguination in our study (Table 1) was in accordance with the results of other authors, who detected from 1.1 to 20.6 mmol/l (EDWARDS et al., 2010), from 4.0 to 19.7 mmol/l (EDWARDS et al., 2010) and from 0.11 to 20.57 mmol/l (HEMSWORTH et al., 2002). The average values of lactate concentrations following both stunning methods can be characterized as those corresponding to high stress, according to HEMSWORTH et al. (2002) and WARRISS et al. (1994). These authors consider a lactate concentration of 4.4 mmol/l as an indicator of low stress, and a lactate concentration of 12.0 mmol/L as an indicator of high stress. In our research, 94.04% of CO_2-stunned and 96.87% electric-stunned pigs showed a lactate concentration higher than 5 mmol/L. Higher values of lactate concentration occurred after electric stunning compared to the CO_2 method, but without statistical significance ($P>0.05$) (Table 2). Both stunning methods might compromise animal welfare and induce stress in different ways and therefore, they are difficult to compare (BRANDT and AASLYNG, 2015).

Meat quality parameters. The effect of stunning method on LD meat quality indicators was only studied in relation to pH45 min, T45 min and pH24 h. Optimal pH values measured 45 minutes after slaughter are greater than 6.1 (DALMAU et al., 2009) and, according to some authors, they range from 6.0-6.7 (HONIKEL, 1998). The average pH45 min in our research was within the optimal range

(Table 1). The initial pH (45 min) of the LD muscles of pigs submitted to electrical stunning was lower ($P<0.05$) compared with those stunned with CO_2 (Table 2), which is in agreement with the results of other authors (MARCON et al., 2019). Electrical stunning leads to physical stress and can accelerate post-mortem muscle glycogen breakdown, leading to a rapid drop in pH. This was confirmed by the significantly higher ($P<0.01$) meat temperature after electrical stunning (39.72°C) compared to CO_2 stunning (38.73°C).

The extent of post-mortem pH decline, which is largely determined by the amount of glycogen in muscles at slaughter, and the ultimate pH level play a key role in the development of drip loss and color (TERLOUW et al., 2021). The optimal values of pH24 h differ slightly among authors, and can be 5.4-5.85 (HONIKEL, 1998), 5.3-5.7 (BRISKEY and WISMER-PEDERSEN, 1961). The pH24 h in our study was lower ($P<0.05$) in pigs electrically stunned compared to those stunned by CO_2 (Table 2), but were within the optimal range (HONIKEL, 1998).

In the current study, the method of stunning did not affect drip loss at 24h or at 48h (Table 2). These results are in agreement with the findings of HAMBRECHT et al. (2004) who, after stressful procedures before slaughter, determined similar values of drip loss at 24h and drip loss at 48h after CO_2 stunning (1.91 % and 2.76 %) and after electric stunning (1.90% and 2.97%), which were not statistically significantly different. The stunning method did not influence meat sensory color, which was in agreement with the findings of HAMBREHT et al. (2004).

Conclusions

Electrical stunning was related to lower meat quality (lower pH45 min, higher T45 min, and lower pH 24 h). Under the conditions of this study, no significant effects of stunning methods on the stress parameters were seen. Stun-quality problems were detected in the abattoir using a dip-lift system, as well as using electrical stunning. Hence, stunning should be well planned and professionally carried out to minimize stress, and thus to assure adequate meat quality.

Financial support statement

The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-47/2023-01/200143).

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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Received: 13 December 2023
Accepted: 16 January 2024
Online publication: 10 May 2024

STAJKOVIĆ, S., D. VASILEV, M. DIMITRIJEVIĆ, N. ČOBANOVIĆ, N. KARABASIL: Utjecaj metode omamljivanja na pokazatelje stresa i kvalitete mesa svinja. Vet. arhiv 94, 297-304, 2024.

SAŽETAK

Ukupno 100 svinja iz komercijalnog uzgoja nasumično je podvrgnuto dvama tretmanima omamljivanja u klaonici: ugljikovim dioksidom (CO_2) i električnim omamljivanjem. Mjereni su učinci na stres svinja i kvalitetu mesa. Određivani su laktat i kortizol u krvi, te postmortalni pH (pH45 min; pH24 h), temperatura (T45 min), sposobnost vezivanja vode i senzorna boja na *longissimus dorsi, pars lumbalis*. Nakon električnog omamljivanja, u usporedbi s omamljivanjem CO_2, pH45 min i pH24 h su se smanjili ($P<0,05$), a T45 min je bila viša ($P<0,01$). Način omamljivanja nije utjecao ($P>0,05$) na sposobnost vezivanja vode 24 h i 48 h *post mortem* i senzornu boju, kao ni na pokazatelje stresa, laktat i kortizol. Međutim, kolebanje pokazatelja stresa kod pojedinačnih životinja unutar klaoničke serije bila je vrlo visoka, a njihove prosječne koncentracije upućivale su na visoku razinu stresa. Iz perspektive dobrobiti i zaštite životinja, obje metode omamljivanja treba pravilno provoditi u skladu s definiranim postupcima kako bi se smanjio stres i osigurala odgovarajuća kvaliteta mesa.

Ključne riječi: svinje; omamljivanje plinom; električno omamljivanje; kvaliteta mesa; stres.