CAN CO_2 STUNNING MEET WELFARE OF SLAUGHTER PIGS?

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SUMMARY

Stunning of pigs using CO_2 is not undisputed as to animal welfare aspects particularly because of too long stun-to-stick times. 460 pigs were subjected to six treatments: 80 vol% CO_2 for 70 s and for 100 s, stun-to-stick interval 25–35 s; the 100 s treatment also stun-to-stick time of 40–50 s; and the same treatments with 90 vol% CO_2 accordingly. All animals were tested for clinical reactions, catecholamines and lactate. Stunning (80% or 90% CO_2) was found to be acceptable for animal welfare only in combination with 100 s exposure times and stun-to-stick times of 25 to 35 s.

Keywords: carbon dioxide (CO_2), animal welfare, stunning, catecholamines, epinephrine, norepinephrine, lactate, corneal reflex

INTRODUCTION

Approximately 240 million pigs were slaughtered in the EU-25 countries in 2006, the most in Germany (49 million), and Spain (38 million) (Polet, 2006). An increasing number of abattoirs across Europe, especially the larger ones, are changing over from electric to stunning of slaughter pigs by carbon dioxide (CO_2). Generally, more pigs per hour can be slaughtered if CO_2 stunning facilities are used. Besides, it is said to result in a better meat quality when compared to electrical stunning. Both stunning methods are legally accepted. Even though the Scientific Committee has considered different scientific opinions (ScVC, 1997; EFSA 2004) on the slaughter and killing of animals since 1993, no basic amendments have yet been made of Directive 93/119/EC (Anonymous, 1993). Although this Directive permits stunning with an atmosphere containing at least 70% CO_2 by volume in air for a minimum of 70 s, the Scientific Committee (ScVC, 1997) has proposed a concentration of at least 80% CO_2, and this has been implemented in different national regulations (for example in Germany) since 1999 (Anonymous, 2004). However, after stunning with 80% CO_2 for at least 70 s animals still show signs of consciousness before exsanguinations. An Opinion Paper of the EFSA (2004) recommends a concentration of 70% to 80% CO_2 at the first stop in the stunning pit and 90% at the bottom in addition to exposure to the gas for more than 100 s. Anyway, not only the CO_2 concentration and the time of exposure to the gas are important factors for animal welfare, but also the time from not being exposed to the gas anymore until sticking of the animals. This stun-to-stick time is recommended (Anonymous, 2004) to be not longer than 30 s after the last stop in the atmosphere or 20 s after the animal is removed from the stunning chamber. In any case, sticking must be carried out before the animal regains consciousness (EFSA, 2004). Throughout Europe slaughter facilities become bigger and more pigs are put into each stunning gondola, especially since the time of exposure has been recommended to be as much as 100 s (EFSA, 2004), this often resulting in long stun-to-stick

intervals for some of the stunned pigs. As gas stunning normally is a reversible stunning method, animals not bleeding to death fast enough after tipping will regain consciousness during the further slaughter procedure.

The objective of the investigation was to determine the impact of different CO_2 stunning procedures and stun-to-stick intervals on animal welfare. In the end the aim was to see if the CO_2 stunning of slaughter pigs can meet animal welfare criteria, meaning in the following that slaughtered pigs are not subjected to a realisation of pain after CO_2 stunning.

MATERIALS AND METHODS

In a commercial abattoir 460 pigs were slaughtered with six different slaughtering methods: slaughter pigs were stunned with two different concentrations of CO_2 ($\geq$ 80% and $\geq$ 90% volume in air) for two different exposure times (70 and 100 s). Stun-to-stick intervals were between 25 to 35 s. Additionally, animals were investigated when stuck 40 to 50 s following the 100 s exposure to the CO_2 atmosphere. In each stunning treatment, two to three (100 s exposure: also four) pigs were brought into the gondola for CO_2 stunning. A one-gondola dip-lift system was used. The motor of the gondola transporter was set to reach the bottom of the pit in 22 s and to return to the ejection level 25 s after restarting there. The actual gas concentrations to which the animals were exposed were determined by an Advance Optima System® (Hartmann & Braun Analysetechnik, Frankfurt a.M., Germany) both inside the gondola at the pigs' nose level and on the bottom of the pit. The animals were shackled and hoisted before exsanguination.

In order to get an overview if the pigs were stunned successfully under each stunning treatment, the depth of unconsciousness was determined by testing clinical reflexes or reactions. Twenty-five to thirty-five seconds after the animals were tipped out of the gondola and immediately after sticking, each pig was tested for a reaction to a painful stimulus on the nasal septum, followed by a digital touch of the cornea and the eye lid (corneal and palpebral reflex), and the auscultation of the heart beat (~ 40 s after tipping, for a duration of 20 s). During the whole time of exsanguination a second person recorded directed movements of the animals. The movements were graded into three categories: 1, negligible (up to five running motions, single head movements); 2, moderate (continuous but moderate running motions, head movements); and 3, profound (massive running motions, recurring movements of the whole body).

Beside clinical reaction, parameters (catecholamines, lactate) in the blood plasma of the slaughtered pigs indicating the realisation of stress were tested. The first sticking blood from each stunned pig was collected into a 10 mL EDTA- tube for haematological use (1.6 mg EDTA/mL blood; Sarstedt, Nümbrecht, Germany) pre-prepared with 250 µL of a stabilising solution consisting of EGTA (ethylene glycol bis-aminoethyl ether N, N, N', N'-tetra acetic acid) and reduced glutathione (both Sigma Aldrich Chemie, Germany) in an aqueous solution of pH 7.0 to 7.5. At exsanguination, the prepared tube was filled with approximately 10 to 15 ml of blood. The collected blood was immediately put on ice and centrifuged upon arrival at the Institute for 10 min at 1,500 x g to extract the blood plasma. Approximately 1.5 ml of plasma was transferred two times to reaction tubes which were immediately frozen at –80°C until analysis. Quantitative detection of norepinephrine and epinephrine from the blood plasma was carried out in the neurobiological laboratories of the University of Göttingen with a high-performance liquid chromatographic (HPLC) method (Musso, Vergassola, Pende & Lotti, 1990) routinely used for human and animal plasma. Di-hydrobenzylamine (10 µL / 10 ng substance) was added to the samples (blood plasma) as an internal standard. After 25 sample measurements a reading with

control plasma standards was integrated in the test procedure. The quantitative detection of lactate (L(+) lactate) was carried out in an Auto-Analyser System® (Bran & Luebbe, Hamburg, Germany) with a continuous-flow method for indirect observation of the enzymatic reaction of $NADH + H^+$ (Stahlhut-Klipp, 1975).

All data was analyzed using the SAS program, version 9.1 (SAS Institute Inc., Cary, NC, USA, 2004). For all animals proportion scaled, quantitative variables (catecholamine values, lactate) and nominal scaled, qualitative variables (clinical reflexes etc.) were detected. The results of the variables of the individual animals of each slaughter day (only one treatment) were tested for significant differences. No statistical differences were calculated between the animals of one day and between animals of one treatment. Conclusively, all animals assessed to one and the same stunning method variation were combined into one group, resulting in six different treatment groups. Every group was subjected to a descriptive and explorative data analysis (Proc. MEANS), and all measurements were tested for Gaussian distribution, and subjected to the Shapiro-Wilk test (Proc. UNIVARIATE). Box-plots used for graphic representation of data were created with Sigmaplot for W!indows 9.01 (SysStat Software, Inc., Point Richmond, CA, USA, 2004). The Wilcoxon's two-sample test and the Kruskal-Wallis test were used for two group significance tests if measurements were non-parametric (Proc. NPAR1WAY), and the t-test if distribution was parametric[0] (Proc. T-TEST). The following levels of significance were defined: $p < 0.05$, significant; $p < 0.01$, highly significant; and $p < 0.001$, most significant[0].

RESULTS AND DISCUSSION

The results of the positive clinical reactions are given in Figure 1. The data on clinical parameters for evaluating the depth of unconsciousness were gathered immediately after hoisting and sticking. The figure gives the relative occurrence in percent of the positive clinical reactions of the animals for each stunning procedure. Overall the most positive answers to stimuli, visible movements or audible heart beats occurred in animals stunned with 80% CO2, especially GC8-70, followed by GC8-100 with a stun-to-stick interval of 40 to 50 s. The same distribution was found with 90% CO2 stunning, with the most positive findings in the GC9-70 procedure, followed by GC9-100/40-50s.

According to Holst (2001) and EFSA (2004) presence of a positive corneal/palpebral reflex in more than 5% of the animals is a sign of an unsuccessful stun. Furthermore, there should be no spontaneous blinking of the eye and convulsions whatsoever, and there should be only brief gagging and gasping. In light of these demands, stunning with 90% (88% to 91%) CO_2 for 100 s will result in successful stunning, whereas the time from stunning to sticking is of minor importance. Gasping and convulsions were observed in pigs especially frequently after stunning with 80% CO_2, most often after 70 s and 100 s exposure in combination with a stun-to-stick interval of 40 to 50 s. There also were high numbers of pigs gasping and with convulsions after stunning with 90% CO_2 for 70 s.

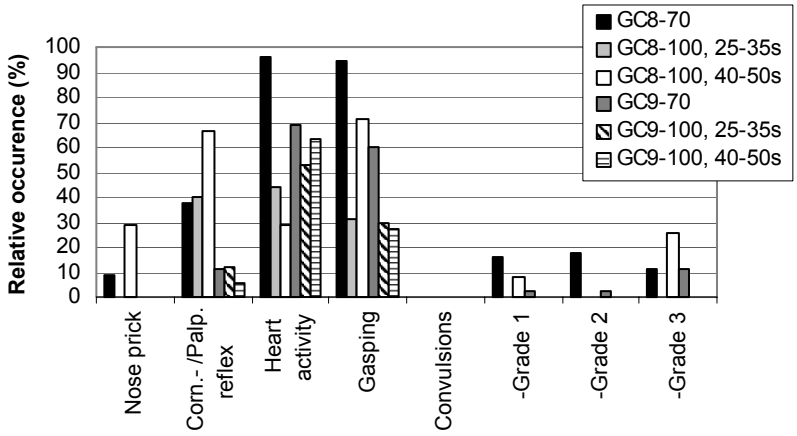

Figure 1. Percentage (relative occurrence) of positive clinical reactions under different stunning procedures: 80% CO_2 by volume in air: GC8; 90% volume in air: GC9, at different duration times in the stunning chamber (70 s, 100 s) and stun-to-stick intervals (25 to 35 s, 40 to 50 s)

Figure 2 shows the results obtained for epinephrine and norepinephrine in a box plot diagramme. There were no statistically significant ($p > 0.05$) differences in epinephrine or norenephrine levels when the animals were stunned with 80% or 90% CO_2 for 70 s or 100 s (GC8-70, GC8-100, etc.) and with stun-to-stick intervals of 25 to 35 or 40 to 50 s (GC8-100/25-35s GC8-100/40-50s, etc.). This was also due to high standard deviations. Total epinephrine values ranged from 149 nmol/L (with GC8-70) to 1721 nmol/L (with GC9-70). The lowest median epinephrine value was found in the GC8-70 experiment and the highest median value in the GC9-100/25-35s experiment. Norepinephrine levels ranged from 133 mmol/L to 3648 mmol/L (both with GC9-70). Norepinephrine median values were lowest with the GC8-100/40-50s procedure, and highest with the GC9-100/40-50s procedure. The ratio of norepinephrine (NEp) to epinephrine (Ep) ($F_{NEp:Ep}$) was about 2.7 for all stunning procedures tested here.

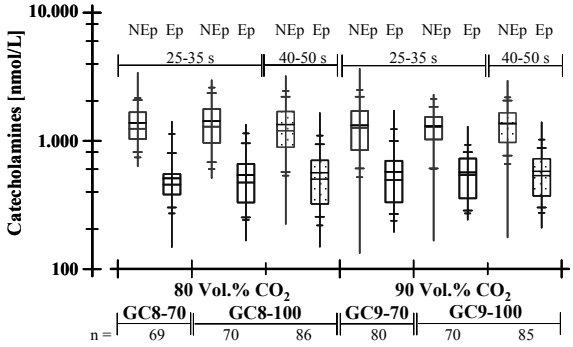

Figure 2. Effect of different CO_2 concentrations (80 vol%: GC8; 90vol%: GC9), different stunning times (70 s, 100 s), and stun-to-stick intervals (25 to 35 s, 40 to 50 s) on plasma concentrations of the catecholamines norepinephrine (NEp) and epinephrine (Ep). Box plot

Figure 3 shows the variation in and distribution of the lactate concentrations in the plasma of the slaughtered pigs. Overall values ranged between 2.41 mmol/L (GC9-70) and 35.8 mmol/L (GC8-70). Lactate levels in calm, rested pigs are said to vary from approximately 0.1 mmol/L blood plasma (Neubert, Gurtler, & Valentin, 1996) to about 2.0 mmol/L (Jensen-Waern & Nyberg, 1993). The broad range in all treatments shows that under every stunning method we seem to get stressed animals. However, the median lactate values after 90% CO_2 stunning were lowest with GC9-70 and highest with GC9-100/40-50s. Stunning methods with 80% CO_2 resulted in higher median lactate values: the lowest values with GC8-100/25-35s and the highest with GC8-70. The differences in lactate levels between all stunning procedures ranged from most significant ($p < 0.001$) to significant ($p < 0.05$). Extremes of over 30 mmol/L lactate were found after stunning for only 70 s both with 80% and 90% CO_2 (Figure 3). When the animals were kept in the atmosphere for 100 s, values were frequently above 20 mmol/L; the highest were 24 mmol/L, which was found with GC8-100/25-35s, and 21 mmol/L, with GC8-100/40-50s. The highest lactate level with the two GC9-100 procedures was 18 mmol/L.

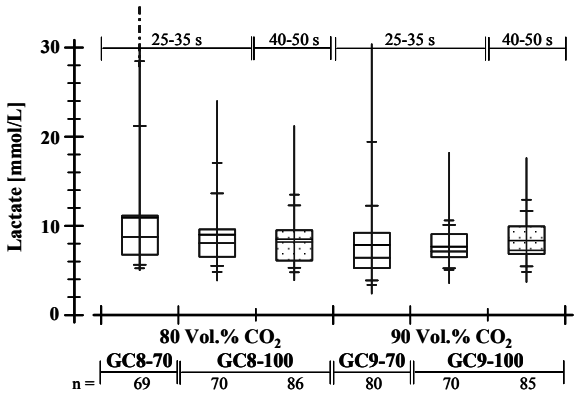

Figure 3. Effect of different CO_2 concentrations (80% by volume in air: 80 Vol.%, GC8; 90% volume in air: 90 Vol.%, GC9) at different stunning duration times (70 s, 100 s) and stun-to-stick intervals (25 to 35 s, 40 to 50 s) on plasma concentrations of lactate. Box plot

In conclusion our results of the testing of clinical parameters show that stunning with 80% CO_2 for 70 or 100 s correlated with high overall numbers of pigs with positive reflex reactions. A stun-to-stick interval of between 40 and 50 s after stunning in 80% CO_2 for 100 s in particular is not acceptable for animal welfare reasons; nor does exposure for 70 s in a stunning chamber with 90% CO_2 result in successful unconsciousness. These findings clearly show the importance of short stun-to-stick intervals for animal welfare. Therefore, the most important combination of factors to ensure sufficiently humane stunning has been said to be long exposure time to the gas (e.g. 150 to 160 s) with a stun-to-stick interval of about 70 to 90 s (EFSA, 2004; Holst, 2001). It has also been recommended that concentrations of 85% to 90% CO_2 be used, with exposure times of 100 to 120 s and stun-to-stick intervals of no more than 30 to 35 s, after exposure for 150 s, stun-to-stick intervals of 60 to 75 s may also be acceptable (EFSA, 2004; Holleben et al., 2002). This was confirmed by our results. Since new gas stunning systems place up to seven or eight pigs in each stunning unit, where stun-to-stick intervals of 70 to 80 s are not unusual (EFSA, 2004), it

is essential that action be quickly taken regarding new legal recommendations in light of these findings.

CONCLUSIONS

The presented results are not able to eliminate doubts if the presently applied CO_2 stunning techniques follow animal welfare. Particularly too long stun-to stick-intervals. Proposals are given with which the stress of the animals can be kept to a minimum within the framework of the existing legal regulations. What appears to be the best for the protection of the animals as well as safeguarding the meat quality is at present a combination of high CO_2 concentration (90 vol%), sufficiently long retention time in the gas atmosphere (100 seconds) and the implementing of the exsanguinations incision at the latest 20 seconds after emission from the stunning pit. Nevertheless, it still appears necessary to "shadow" carbon dioxide stunning critically both experimentally and in the practice in order to improve the protection of animals in this extremely sensitive socio-political area. Last but not least, this is one of the important veterinary tasks in the abattoir.

ACKNOWLEDGEMENT

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