It is our understanding that the walls used in the stunning unit are partly covered with metal, and a motor is placed above the crate containing the pigs. Under such conditions, it is likely that the signals from surface electrodes as described would be disturbed. Hence, the interpretation of results may be difficult.

Measurements of Burst Suppression (BS) may be a more robust indicator. However, it is likely that the animals lost consciousness *before* BS started to increase.

Rodriguez states that "a corneal reflex… has been described as the first reflex to disappear during *induction* to unconsciousness with CO_2…". Classical schemes on gas narcosis state that the corneal reflex is supposed to disappear in the second to fourth level of the third phase of narcosis, whereas consciousness is lost during the first phase of induction (called STI in Table 1).

Rodriguez's technique to obtain the electroencephalographic (EEG) signal and the middle latency auditory signal (AEP) is very similar to that carried out previously by Martoft (Martoft *et al* 2001, 2002) and the work is done in collaboration with the same EEG/EP research collaborators (EW Jensen and B Rodriguez). Extraction of the AEP signal from the raw EEG trace requires averaging. This is done with the use of autoregressive modelling with an exogenous input to make the AEP trace as close to 'real time' as possible. The depth of anaesthesia index used by Rodriguez is calculated from autoregressive modelling with exogenous input (DAI = AAI), and it is similar to the method used by Martoft. In Rodriguez's work, the time resolution was one second (number of sweeps for averaging within this timeframe is not described). In Martoft's work, it was 1.7 s based on an average of 15 sweeps, each of 0.11 s. Martoft found that it took at least 15 sweeps to have a sufficiently strong AEP signal to be able to subtract it from the raw EEG during the period of CO_2-anaesthesia induction. During CO_2 inhalation, Rodriguez compared the AEP averaged over a number of sweeps (number not defined) retrieved over one second to the AEP signal retrieved prior to CO_2 inhalation. This should show the difference in AEP between a pig being awake, non-CO_2 influenced, and later on under CO_2 influence. However, the short recording time (one second) must have given predicted AEP signals with a great deal of variability. This could be the reason for Rodriguez not finding a gradual change towards depressed AEP signals in the early part of the exposure time as described by Martoft. Besides, the difference in the depths of anaesthesia indexes during the early part of the exposure time and the value of the indexes from later time-points (later than 50 s) correlates very well between Rodriguez and Martoft.

Conclusion

In view of the comments above, we doubt that the work performed by Rodriguez *et al* (2008) can justify the quite 'stunning' conclusion, that consciousness is not lost until after 60 seconds of exposure to 90% carbon dioxide, and that these 60 seconds are filled with strongly aversive behaviour. If it turns out that Rodriguez's results are based on 'sound scientific work' in spite of our doubts, it would have a huge impact on recommendations and regulations regarding animal welfare in slaughter plants. Hence, we would appreciate a comment on these topics.

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Response to Tolo, Christensen, Martoft and Forslid's letter 'CO_2-stunning in pigs'

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We would like to thank you for giving us the opportunity to make a few comments and clarifications in response to the letter by Mr Elisiv Tolo, Mr Leif Christensen, Dr Lotte Martoft and Dr Anders Forslid. Although the authors of the letter refer to papers for which they do not give a full reference, we would try to address all the points they raise.

The statement that CO_2 concentrations above 80% cause less reaction than lower concentrations is neither demonstrated in the EFSA report nor in Raj and Gregory's papers (1995, 1996). To support this, the authors of the letter refer to Raj and Gregory (1995). However, the conclusion of this paper is "that 90% CO_2 in air in which the induction of anaesthesia is rapid and respiratory distress is severe but short-lasting".

We agree with the authors of the letter that group stunning at high CO_2 concentration has certain animal welfare advantages compared with electrical stunning. Pigs are stunned in groups with minimum levels of restraint and handling stress. However, when pigs are exposed to high concentrations of CO_2, loss of consciousness is not immediate and pigs may experience aversion during exposure to the gas (Raj & Gregory 1995). We think that research is needed to find a non-aversive gas mixture that can be used in the

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group-wise stunning system. We believe that the increase in popularity of the CO_2-stunning systems might be more likely to be due to their positive effects on meat quality rather than to improve animal welfare.

Velarde *et al* (2007)

This experiment was performed in a commercial stunning system set up and verified by Butina Aps. It is not clear to what the author of the letter is referring with the statement "we assume that no mixing of the gas content in the well occurred during the experiments". The CO_2 concentration at the exposures levels (170 and 113 cm) was not monitored continuously. However, as it is described in *Materials and methods*, the required CO_2 concentration was monitored continuously via sensors fitted on the wall 50 cm above the floor level. When the CO_2 concentration dropped below a pre-set value, a valve released sufficient gas to restore the original setting before shutting off. Therefore, we always refer to treatments according to the gas concentration monitored 50 cm above the floor level (90 vs 70%). We assume that the higher the concentration at this level, the higher the concentration in the intermediate positions, according to the results of the measures taken at the start and at the end of each treatment day. Furthermore, we found a treatment effect (90 vs 70%) on the measures of aversion and loss of posture assessed, also indicating an effect of the CO_2 concentration at these levels (170 and 113 cm) on the aversion and loss of posture. In a paper in the most recent edition of *Animal Welfare*, entitled *Stunning pigs with different gas mixtures: gas stability* by Dalmau *et al* (2010), the stability and uniformity of 90% CO_2 when the cradle was stationary was assessed. The results indicated that the mean decrease of CO_2 after 10 min at these levels is around 2%.

During the behavioural assessment, the observers distinguished easily between escape attempt (according to the definition) and involuntary movement. However, as electroencephalography was not carried out in this study, the level of consciousness during the exposure to CO_2 could not be determined.

We agree with the authors of the letter that several papers state that loss of posture is a sign of unconsciousness. However, none of the electroencephalographic analysis performed in these papers could determine precisely when the animals lost consciousness, and the level of unconsciousness when the pigs lost posture.

Rodriguez *et al* (2008)

The recovery of the surgical operation depends on the surgery itself. In our study, the surgery consisted of a simple puncture into the carotid artery to place a catheter. This surgery was less invasive than the procedures used in previous papers for the collection of blood samples. In fact, the following day, no significant difference was observed in the EEG activity between the individuals with and without a catheter.

The required CO_2 concentration was supplied through an inlet valve at the bottom of the well, and monitored continuously via sensors fitted on the wall 50 cm above floor level. When the CO_2 concentration dropped below a pre-set value, a valve released sufficient gas to restore the original setting before shutting off.

Pigs were exposed to 90% CO_2 in 23 s as described in the paper. The aim of the study was to simulate the conditions of CO_2 stunning in a commercial dip-lift system, where the animals take this amount of time to be exposed to high concentrations of CO_2. Other publications (Martoft *et al* 2003) exposed the animal immediately. The different procedures used in both studies should be taken into consideration when the results of both papers are compared.

Obviously, the greater the number of blood samples taken, the more accurate the curve. However, we believe that the number of blood samples taken was appropriate for our purposes.

In the letter, there is an inconsistency in the Martoft *et al* reference. In the text, it is referred as Martoft *et al* (2003) while in Figure 1 it is Martoft *et al* (2002). We believe that the delay in the increase of arterial pCO_2 during inhalation of CO_2 compared to the results of Martoft *et al* and Forslid and Augustinsson (1988), is due to the different procedures in the exposure to the CO_2. In our study, pigs were not exposed immediately to 90% CO_2 as in the previous works. Our aim was to simulate the commercial conditions of exposure to CO_2 in a dip-lift stunning system, where the animals take 23 s to be exposed to 90% CO_2.

The time to loss of posture is not given because it could not be assessed in animals placed in a net restrainer with the limb 10 cm above the ground. Therefore, the time to loss of posture was not correlated with brain activity, as wrongly suggested in the letter. Also, it could not be compared with the results of Velarde *et al* (2007) as the exposure times were different as well.

The A-line depth of anaesthesia monitor is designed to work in a hostile electrical environment. It is equipped with algorithms for rejection of electrical artefacts, such as those arising from the diathermy, which is of much higher amplitude than that produced by the motor for the lift. Besides, if noise level supersedes necessary signal level then the index number (AAI) will not be calculated.

BS is probably not more robust than the index because it requires a suppression period, which is like a noise-free, almost iso-electrical EEG. But the presence of BS confirms that the noise level is low, otherwise there could not be registered BS.

We agree that it is possible that the animals could lose consciousness before BS starts, but the moment for loss of consciousness is decided by the index and not the BS level.

The paper states that the absence of a corneal reflex is used commercially to assess the effectiveness of stunning. Holst (2001) concluded that from all the reflexes assessed in commercial conditions, the corneal reflex is the first to disappear during induction to unconsciousness with CO_2 and the first to reappear during recovery. Other reflexes, such as cilia (eyelash) reflex, regular respiration, excitation, nystagmus (horizontal vibrating movements of eyeball), and spontaneous blinking of the eye appeared later than the corneal reflex.

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The authors of the letter are misinterpreting what 'resolution' means. The A-line AEP monitor displays a new value on the display every second but the processing time is still based on the ARX model which needs at least 15 sweeps to extract the AEP. This model is identical in the work of Martoft and Rodriguez, although the device version used in the work of Rodriguez is significantly newer than in the work of Martoft, hence more reliable in general, and specifically equipped with a better artefact rejection algorithm. A moving time average is applied in both versions of the AEP monitor which means that the total delay, already included in the calculation, is 6 s.

Conclusion

The conclusions of both papers are based on the results of the studies. In the work performed by Rodriguez *et al* (2008) we concluded that before loss of consciousness, pigs exhibited side-to-side head movements, sneezing, gasping, muscular excitation and vocalisations. All these signs show that induction to CO_2 anaesthesia is not immediate and pigs suffer from fear, pain and/or stress during immersion into gas. These conclusions are also supported by Raj and Gregory (1995, 1996), and the EFSA report (2004). We do not use the subjective term 'strongly aversive behaviour' that is an interpretation of the authors of the letter. We do not try to judge its commercial use from an animal welfare point of view, as this is the task of the policy-makers. In fact, our sole aim was to publish the results of our experiments.

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