Letters

CO_2-stunning in pigs

E Tolo*†, L Christensen‡, L Martoft§ and A Forslid#

† Animalia, Norwegian Meat Research Centre, Norway

‡ Danish Meat Research Institute, Denmark

§ Integrative Pharmacology, Biosciences, AstraZeneca, Lund, Sweden

Lund University, Sweden

* Contact for correspondence: elisiv.tolo@animalia.no

With interest we have read two articles on CO_2-stunning published in *Animal Welfare* in 2007 and 2008. These are: Velarde *et al* (2007), *Aversion to carbon dioxide stunning in pigs: effect of carbon dioxide concentration and halothane genotype* and Rodriguez *et al* (2008), *Assessment of unconsciousness during carbon dioxide stunning in pigs*.

It is our understanding that all researchers agree that CO_2 is aversive to pigs. However, it has been demonstrated that concentrations below 30% and above 80% cause less reactions than concentrations inbetween (Raj & Gregory 1996). In addition, the time period where the animals experience stress or pain decreases with increased concentrations of CO_2. Based on earlier work, (eg Forslid 1992; Raj & Gregory 1996; Martoft 2001, 2002, 2003), it has been concluded that pigs exposed to high concentrations of carbon dioxide lose consciousness within 13–20 seconds after the onset of exposure. The above researchers have all interpreted loss of posture as an early sign of loss of consciousness. Loss of posture may be preceded by uncoordinated movements. Hence, to improve animal welfare at slaughter, the meat industry has been recommended to invest in CO_2 equipment with group-wise stunning at high CO_2 concentrations (> 90%).

The two articles from Velarde *et al* (2007) and Rodriguez *et al* (2008), published in *Animal Welfare*, suggest that earlier conclusions may be wrong. If so, this could have a huge impact on slaughter practices. However, according to descriptions given in the two papers, it is our opinion that the trial conditions, the results obtained and the conclusions drawn are open to interpretation. Below, we raise a number of points.

Velarde *et al* (2007)

Experimental procedure and observations

The experiments were performed in an old dip-lift stunning unit from Butina. The required CO_2 concentration was supplied through an inlet valve at the bottom of the 260-cm deep well, and monitored continuously via sensors fitted on the wall, 50 cm above floor level. During exposure to CO_2, the pigs were only lowered to 170 and 113 cm. The gas concentration in these intermediate positions was only measured at the start and the end of each treatment day. We assume that no mixing of the gas content in the well occurred during the experiments, as the pigs were allowed to take 10 minutes on entering the crate. Hence, it is very likely that the gas concentrations in the well varied quite considerably, and the actual exposure levels in each experiment are not known.

The definition of escape attempts differs slightly from the definition used by Raj and Gregory (1996) who designated thus, "when a pig raised its forelegs on the side of the well either prior to, or at the time it was losing its posture". This compares with Velarde *et al* (2007) and, "*pigs running across the stunning box and sometimes* raising their forelegs on the side of the wall of the crate either prior to or at the time they were losing their posture".

Velarde's definition may challenge the observer when deciding whether an animal is trying to escape or merely losing muscular control as unconsciousness set in. In Velarde's results, escape attempts occur 2.6–3.9 seconds prior to loss of posture. As the narcotic effect of CO_2 is due to a gradual decrease of pH in the brain, it is likely that the animals undergo a reduced level of consciousness 3 seconds prior to the loss of consciousness.

Velarde *et al* (2007) state that loss of posture is a sign of unconsciousness and, from their work in 2007, it can be concluded that 96% of commercial crossbred pigs in a commercial dip-lift system lost consciousness during 21 s exposure to 85% CO_2 gas. This differs markedly from the conclusions drawn in Rodriguez *et al* (2008), where Velarde is a co-author.

Rodriguez *et al* (2008)

Undergoing surgical operation one day prior to testing is not appropriate (the normal procedure is to allow for a postoperative phase of 5–7 days).

Information on gas measurements and gas gradient are missing. See comments regarding Velarde *et al* (2007).

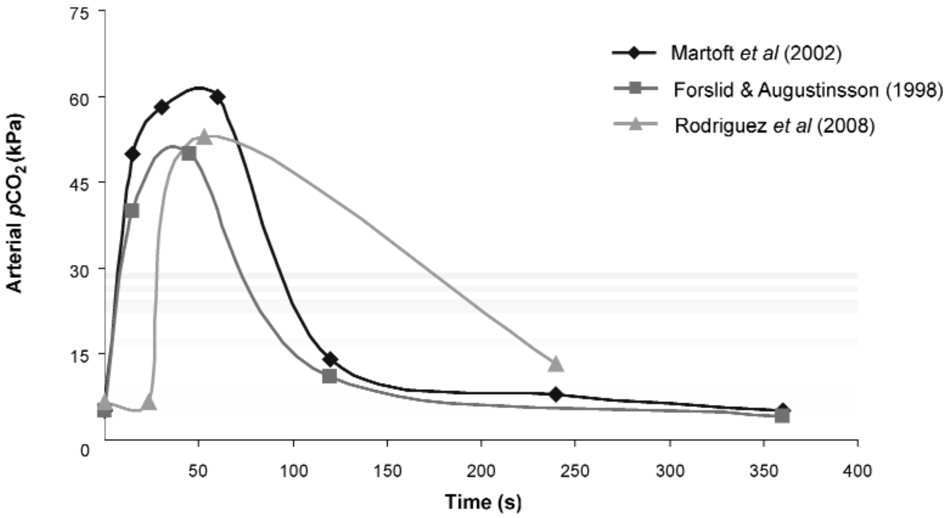
During the first 4–5 seconds of descent only low concentrations of CO_2 are to be expected, hence the gas is neither aversive nor effective during this period. As the gas gradient in this experiment is not known, it is difficult to say when the pigs were exposed to higher concentrations of CO_2. Hence, measuring time-intervals from the start of descent do not provide accurate information.

It is not described how the extended catheter was rinsed to ensure that blood samples represented the actual values at the given times.

The number of blood samples seems rather low.

Compared to other work on CO_2 anaesthesia in pigs, there seems to be a delay in the increase of arterial $p CO_2$ during inhalation of CO_2 (see Figure 1). It is likely that this is due to a potential fault in the experimental blood-sampling technique. A possible explanation for this difference is that the catheters were not rinsed of residual blood prior to sampling as this can cause a delay in measured change in arterial $p CO_2$. A comparison of other blood values reported

Figure 1



The change in arterial pCO_2 during and after exposure to CO_2.

Table 1 Symptoms during the different stages of anaesthesia.

	ST1	ST2	ST3				ST4
			Level 1	Level 2	Level 3	Level 4	
Hearing	+++	++	lost				
Laryngeal reflex	+++	+++	lost				
Sensorium	+++	+/–	lost				
Cough reflex	+++	++	+	lost			
Palpebral reflex	normal	+++	+	lost (normally)			
Corneal reflex	+++	+++	+	+/–	lost	lost	
Light reflex	++	+++	+	+	+/–	lost	
Lacrimation	+++	+++	++	+	+/–	lost	
Limb muscle tone	++	++++	+++	++	+	lost	
Jaw tone	++	++++	+++	++	+	lost	
Respiratory rate	increased		progressive disease			slow/irregular	Ceased, gasping may occur

ST1: analgesia; ST2: excitation; ST3: anaesthesia (4 levels); ST4: paralysis.

in the article with findings from Martoft *et al* (2003), supports this theory, as explained in the next two points.

The pH of the blood decreases rather slowly, due to buffer systems. However, after 15 seconds, Martoft found that this had decreased to 7.15, whereas Rodriguez still had a pH of almost 7.4, 23 seconds after the start of exposure. Even taking into account that the animals were hardly subjected to high concentrations of CO_2 in the first 5–10 seconds of exposure, a pH reduction would be expected in a sample representative for 23 seconds.

Due to the use of different measurement methods (saturation vs mm Hg) it is difficult to compare O_2 saturation in different studies. However, Rodriguez found that pO_2 continued to decrease after the end of exposure, whereas Martoft found that pO_2 started to increase immediately. Given that Martoft's figure gives a correct picture of the pO_2, Rodriguez's results indicates a delay in his values of approximately 15 seconds.

Time to loss of posture is not given.

Based on the depth of anaesthesia index, Rodriguez concludes that in a commercial dip-lift system unconsciousness occurs in pigs after, on average, 60 s exposure to 90% CO_2 gas. It seems strange that Rodriguez and Velarde (co-author to the work) do not discuss the early onset of loss of posture as described by Velarde previously (Velarde *et al* 2007).

https://doi.org/10.1017/S0962728600001834 Published online by Cambridge University Press

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.

References

Forslid A 1992 Muskelkrämpfe während der CO_2-Betäubung von Schweinen vor der Schlachtung: etische Gesichtspunkte. *Fleischwirtschaft 72*: 194-195

Martoft L, Jensen EW, Rodriguez BE, Jørgensen PF, Forslid A and Pedersen HD 2001 Middle-latency auditory evoked potentials during induction of thiopentone anaesthesia in pigs. *Laboratory Animal 35*: 353-363

Martoft L, Lomholt L, Kolthoff C, Rodriguez BE, Jensen EV and Jørgensen PF 2002 Effects of CO_2 anaesthesia on central nervous system activity in swine. *Laboratory Animal 36*: 115-126

Martoft L, Stødkilde-Jørgensen H, Forslid A, Pedersen HD and Jørgensen PF 2003 CO_2 induced acute respiratory acidosis and brain tissue intracellular pH: a ^{31}P NMR study in swine. *Laboratory Animal 37*: 241-248

Raj ABM and Gregory NG 1996 Welfare implications of the gas stunning of pigs 2. Stress of induction of anaesthesia. *Animal Welfare 5*: 71-78

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

A Velarde*†, X Manteca‡ and E Weber§

† IRTA, Finca Camps i Armet, E-17121 Monells (Girona), Spain

‡ Universitat Autònoma de Barcelona, Spain

§ Morpheus Medical Ed, Spain

* Contact for correspondence: antonio.velarde@irta.cat

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