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Relationship between the response to the corneal reflex (depth of narcosis) and specific parameters in the slaughter blood of pigs narcotised with CO_2

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Abstract

There has been insufficient research into CO_2 stunning with regard to its effect on pigs being slaughtered. This lack of knowledge may be at least partly responsible for the partial rejection of CO_2-stunning methods. During routine slaughter work, 598 pigs (average carcase weight: 94 kg) were evaluated. The stunning procedure was carried out in industrial stunning chambers with 90% CO_2 by volume and an exposure time of either 120 or 90 s. The corneal reflex response was evaluated immediately prior to bleeding in order to determine the depth of narcosis. Blood was taken at slaughter (slaughter blood) to determine the partial pressure of breathing gases and the acid-base status. We found that CO_2 stunning mainly produced hypoxaemia, but also normo- and hyperoxaemia, in arteriovenous slaughter blood. No further positive reflex responses occurred at a pO_2 threshold of $\leq$ 1.6 kPa. PCO_2 increased to values of 40 kPa and above. This extreme hypercapnia resulted in a decrease of the slaughter blood pH with values of less than 7.00 (ie, strong respiratory acidosis). Starting with threshold values from $pCO_2 \geq$ 23 kPa and pH $\leq$ 6.85, stunned pigs revealed only a few or no positive reflex responses, respectively. The non-respiratory Stewart-variable serum [SID_3] was elevated to alkaline values of 65 mmol L^{-1} and above, in comparison to the normal values of 45 (± 2) mmol L^{-1}. We conclude that the use of cut-off points such as the pH and/or pO_2 in routine sampling of slaughter animals (eg by application of ion-sensitive electrodes) would establish the depth of narcosis in pigs destined for slaughter. The efficiency of monitoring could thereby be improved during slaughter, in line with the demands of animal welfare.

Keywords: *acid-base parameter, animal welfare, blood-gas tension, CO_2 stunning, corneal reflex response, pigs*

Introduction

Throughout the European Union (including Germany) gases are being increasingly used to stun pigs for slaughter (Cantieni 1977; Forslid & Augustinsson 1988; Raj 1999; Troeger & Moje 2000; Martoft 2001; Buschulte *et al* 2007). According to Nowak and Hartung (2006), more than 30% of all abattoirs use CO_2.

It is well accepted that (warm-blooded) animals must be sufficiently stunned, before they are bled and slaughtered (Corbach 2006; German Animal Protection Law, 24. 07.1972, last amendment 18.12.2007). This implies that large quantities of animals have to be narcotised within seconds and should only be subjected to stress that is unavoidable. Furthermore, the (reversible) loss of consciousness must be sufficient to avoid suffering (German Animal Welfare Slaughter Regulation, 03.03.1997, last modification; 13.04.2006). Moreover, the produced meat has to be of acceptable quality.

In the literature, the extent to which CO_2-stunning technique meets these requirements has been the subject of much deliberation. Nevertheless, consent has been given for CO_2 use in stunning (Erhardt *et al* 1989; Barford 1990; Lagerweij 1990; Troeger & Moje 2000; Martoft *et al* 2002). However, Raj *et al* (1997) and Machold *et al* (2003a,b) are critical of the use of CO_2 and advise against it, on the grounds of animal welfare. Moreover, Jaresch (2001) also describes CO_2 narcosis as unacceptable.

One reason for this difference of opinion might be our poor knowledge concerning the effect of aerogen-induced (ie absorbed by air) CO_2 excess on the physiology of the pig. To broaden and improve our knowledge of this matter, we sought the answer to the following question: are there selective parameters in the slaughter blood of pigs, as assessed by the reflex response, that enable conclusions to be drawn with regard to the depth of narcosis?

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Table 1 Parameters relating to CO_2 exposure during stunning of pigs and results of testing for corneal reflex responses.

Parameter	Abattoir I	Abattoir II
CO_2 content in industrial stunning chamber (% volume)	90	90
Exposure time in CO_2 chamber (s)	120	90
Range in time between release from CO_2 chamber and bleeding/sample collection (s)	69–101	28–35
Total number of pigs (n)	2,650 (100%)	2,100 (100%)
Number with positive response (n)	163 (6.2%)	360 (17.1%)
Number with negative response (n)	2,487 (93.8%)	1,740 (82.9%)

Materials and methods

Study animals

This study included 598 pigs from two different abattoirs: abattoir I (located in west Germany) with n = 254 (42.5%) and abattoir II (north Germany) with n = 344 (57.5%). Pigs for slaughter originated from 128 different farms in the general vicinity of each abattoir. Pre-slaughter examination confirmed their suitability for the regular slaughter method. Post mortem carcase weight reflected the relative homogeneity in this pig population, with an average value of 94 kg.

Abattoirs and stunning

In abattoirs I and II, the daily slaughter numbers were 20,000 and 3,500 pigs, respectively. In abattoir I, it was a '2+1 backloader XL 6 system' (Butina ApS, Denmark) that was used to transport the pigs into the CO_2 chamber while in abattoir II it was the 'combi type' (Butina ApS, Denmark). The CO_2-stunning procedure varied to a certain extent between abattoirs I and II (see Table 1). The technical details of the slaughter process are not relevant to the evaluation of the measured parameters and are therefore not included in the study.

Animals were transported by a band conveyor after release from the CO_2 chamber. Pigs were exposed to normal air conditions (O_2 = 20.9% by volume, CO_2 = 0.03% by volume) during this time. Animals were shackled and hoisted and taken to the bleeding position. The processing speed varied between abattoirs (see Table 1). The stage of narcosis was evaluated through assessment of the corneal reflex immediately prior to bleeding. A positive reflex response was denoted by any level of eyelid closure or blinking when the cornea was touched. The cornea was touched only once for logistical reasons. In the case of no reaction, the reflex response was determined as being negative.

Sample collection and laboratory analysis

The analysis was carried out during routine slaughtering and, as such, not every pig slaughtered could be included in the study. Every fifth to tenth (randomly distributed) animal was marked with a plastic ear-tag (Mini-Neoflex, Trapez white, Hauptner, Herberholz, Solingen, Germany). These ear-tags were used for the further identification of carcases and to assign the obtained samples in the ongoing slaughter process (Rindermann 2008).

The exsanguination of stunned pigs was performed by using custom-made tubular-shaped knives (Anitec A/S, Nörressundby, Denmark). The tubular shaft was connected to a hose system in order that the study animal in question could have blood collected by vacuum suction.

The collection of blood samples to determine blood gases and acid-base status was carried out in accordance with international standards (Burnett *et al* 1995). Samples were taken directly from the puncture wound. Blood samples were drawn by heparinised plastic syringes (1.0 ml, Klinika Medical GmbH, Germany) and syringes were immediately sealed with a rubber cap and stored in ice prior to analysis. Within 30 min, blood samples were analysed to determine pO_2, pCO_2 and pH values via a blood gas analyser (ABL 605, Radiometer, Copenhagen, Denmark). For the analysis, the pigs' standardised body temperature was considered to be 39.0°C.

Additionally, serum was collected in 2 ml Eppendorf tubes, subsequently deep frozen (–18°C) and stored (≤ 8 weeks). Serum samples were used to determine sodium, potassium and chloride values via ion-sensitive electrodes. Based on these serum electrolyte data, we calculated the 'strong ion difference' (SID), according to the formula serum $[SID_3]$ mmol L^{-1} = $[Na^+]$ + $[K^+]$ – $[Cl^-]$ mmol L^{-1}.

Statistical analysis

The SPSS program (version 16.0 for Windows) was used to process data, including graphical representation of the results. A receiver operating characteristic (ROC) analysis was carried out as a reference (MedCalc® Program, version 10.0 edited by Frank Schoonjahns) to determine the diagnostic valence of the parameters. This method is based on the concept of predictive assessment of a test result with the help of a 'cross tabulation' (Table 2; Farver 2008; Stockham & Scott 2008).

We generated predictive values by calculating probabilities so that the diagnostic quality of test results could be verified (Table 2). Cut-off points between a particular criterion of the slaughter blood and the response to the corneal reflex were determined based on the ROC analysis. In this study, it was important to identify stunned pigs with positive reflex response as accurately as possible. Their depth of narcosis was found to be insufficient. The result of sensitivity is deemed therefore to be more biologically valuable (Table 2: true positive non-stunned pigs) than the result of specificity (Table 2: true negative stunned pigs) (Farver 2008; Stockham & Scott 2008). Cut-off points with high sensitivity, eg 100%, were determined based on the ROC analysis and noted for further application. The values of sensitivity and specificity act in the opposite direction, ie high sensitivity values are associated with low values of specificity and *vice versa*.

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Table 2 Matrix for categorising test results (TP, FP, TN, FN) and the calculation of some predictive values.

Criterion	Test: Reflex response		Total
	Positive	Negative	
Non-stunned	True positive (TP)	False negative (FN)	TP + FN
Stunned	False positive (FP)	True negative (TN)	TN + FP
Total	TP + FP	TN + FN	TP + TN + FP + FN

Diagnostic sensitivity (%) = TP/(TP + FN) × 100.
A test showing high diagnostic sensitivity (few FN results) is good for screening the presence of non-stunned animals.
Diagnostic specificity (%) = TN/(TN + FP) × 100.
A test showing high diagnostic specificity (few FP results) is valuable for confirming that an animal is stunned.

Table 3 Data (median [95% confidence interval]) of ROC analysis for various slaughter blood criteria and the response to the corneal reflex of pigs narcotised with CO_2 (n = 530 with a negative response and n = 68 with a positive response).

Criterion: Slaughter blood parameter	AUC*	Cut-off point	Sensitivity (%)	Specificity (%)
PO_2	0.69 (0.65–0.72)	3.1 kPa[a]	72 (60–82)	59 (55–64)
		1.6 kPa[a]	100 (95–100)	11 (8–14)
PCO_2	0.67 (0.63–0.70)	20.1 kPa[a]	78 (66–87)	50 (46–54)
		41.9 kPa[a]	100 (95–100)	0 (0–1)
pH	0.68 (0.64–0.72)	6.97[a]	69 (57–80)	61 (56–65)
		6.77[a]	100 (95–100)	4 (3–6)
Na^+	0.55 (0.51–0.59)	147 mmol L^{-1}[a]	24 (14–35)	89 (86–91)
		129 mmol L^{-1}[b]	100 (95–100)	2 (1–4)
K^+	0.55 (0.51–0.59)	9.9 mmol L^{-1}[a]	22 (13–34)	92 (89–94)
		21.0 mmol L^{-1}[b]	100 (95–100)	2 (1–3)
Cl^-	0.62 (0.58–0.66)	97 mmol L^{-1}[a]	74 (61–84)	51 (46–55)
		89 mmol L^{-1}[b]	100 (95–100)	6 (4–8)
Serum [SID_3]	0.60 (0.56–0.64)	52.9 mmol L^{-1}[a]	26 (17–39)	92 (89–94)
		63.4 mmol L^{-1}[b]	100 (95–100)	6 (4–8)

* Evaluation of area under curve (AUC) according to Greiner *et al* (2000): 0.5 = no predictive value; 0.5 < AUC ≤ 0.7 little predictive value; 0.7 < AUC ≤ 0.9 moderately predictive; 0.9 < AUC < 1 highly predictive; AUC = 1 perfect predictive power.
[a] Cut-off point 1: highest accuracy meaning minimal false negative and false positive results.
[b] Cut-off point 2: defined by a sensitivity of 100% with maximal specificity.

Two different cut-off points are given; cut-off point 1 corresponds with the highest accuracy, meaning minimal false negative and false positive results while cut-off point 2 is defined by a sensitivity of 100% with the maximal specificity (Table 3).

Results

The relationship between specific parameters in the slaughter blood and the reflex response is illustrated in scattergrams.

Compared with the physiological findings in the arterial and venous blood, the CO_2 excess provoked a decrease in the pO_2 values of the slaughter blood in the majority of pigs (Figure 1; normal values: striped area). However, a number of animals showed normal or increased pO_2 values. The cut-off point 1 at 3.1 kPa emerged as the parameter with predictive values of sensitivity = 72% and specificity = 59% (Table 3). A threshold value of 1.6 kPa reflected a sensitivity of 100% (cut-off point 2).

Of the included slaughter pigs (n = 598), 334 (100%) reached the cut-off value of 3.1 kPa with 19 (6%) giving a positive response and 315 (94%) giving a negative response to the tested corneal reflex. At the lowest threshold value of $pO_2 \leq 1.6$ kPa (cut-off point 2), all 58 stunned pigs showed a negative response to the corneal reflex (Figure 1).

CO_2 stunning provoked a highly variable increase in pCO_2 in the slaughter blood, up to values of ≥ 40 kPa, in comparison with the physiological findings (Figure 2). ROC data analysis determined cut-off point 1 to be 20.1 kPa with an average sensitivity/specificity of 78/50% (Table 3). A pCO_2 value of ≥ 20.1 kPa was reached by 286 (100%) of the stunned pigs, with 17 (6%) giving a positive response and 176 (94%) a negative response to the corneal reflex. In 180

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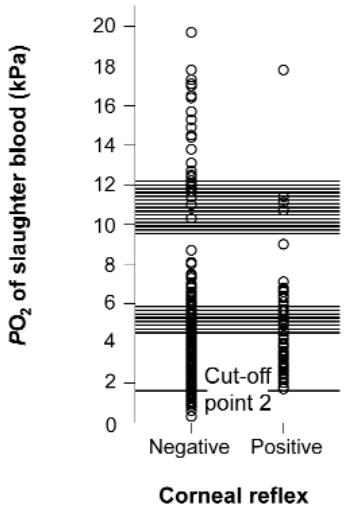
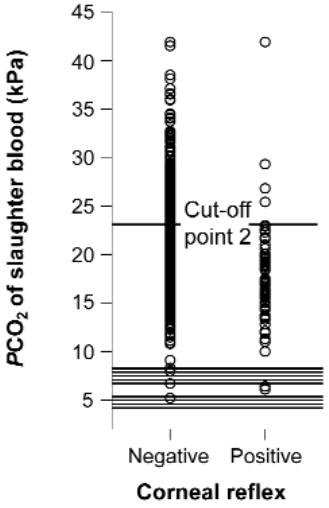
Figure 1



Scattergram for pO_2 (kPa) values in slaughter blood (mixed arteriovenous blood) and tested response to corneal reflex immediately before bleeding in pigs narcotised with CO_2 prior to slaughter (n = 598; negative = 530, positive = 68 animals). Normal values (striped area): $P_{arterial}O_2$ = 9.7–12.2 kPa, $P_{venous}O_2$ = 4.3–6.0 kPa.

Figure 2



Scattergram for pCO_2 (kPa) values in slaughter blood (mixed arteriovenous blood) and tested response to corneal reflex immediately before bleeding in pigs narcotised with CO_2 prior to slaughter (n = 598; negative = 530, positive = 68 animals). Normal values (striped area): $P_{arterial}CO_2$ = 4.7–5.6 kPa, $P_{venous}CO_2$ = 5.9–7.3 kPa.

(100%) of the narcotised pigs, a valuation level for pCO_2 in slaughter blood of 23.0 kPa or more was obtained (Figure 2); of these, 4 (2%) showed a positive and 176 (98%) a negative reflex response.

CO_2 narcosis led to a variable decrease of the pH value in the slaughter blood (Figure 3). At cut-off point 1, the ROC analysis identified a pH of 6.97 with a sensitivity/specificity of 69/61%. An extreme cut-off point of pH ≤ 6.77 coincided with an average sensitivity of 100% (Table 3). A slaughter blood pH of ≤ 6.97 was reached in 342 (100%) of the stunned pigs; of these, 21 (6%) showed a positive and 321 (94%) a negative corneal reflex. With a preconditioned pH value of ≤ 6.85, the corresponding 91 (100%) pigs consisted of one case (1%) showing a positive reflex response and 90 (99%) exhibiting a negative response (Figure 3).

In stunned pigs, serum $[SID_3]$ predominantly increased related to the reference interval (Figure 4). Both positive and negative reflex responses were observed over a wide range of increased $[SID_3]$ values. ROC analysis determined a serum $[SID_3]$ value of 52.9 mmol L^{-1} as the cut-off point 1. Serum $[SID_3]$ values from ≥ 52.9 mmol L^{-1} were measured in 592 (100%) of the stunned pigs, with 51 (9%) and 491 (91%) revealing positive or negative responses, respectively. An extreme value (cut-off point 2: ≥ 63.4 mmol L^{-1}) was accompanied by a sensitivity of 100% (Table 3). A serum $[SID_3]$ from ≥ 63.4 mmol L^{-1} was reached by a total of 31 (100%) animals, with one case (3%) showing a positive response and 30 cases (97%) showing a negative reflex response (Figure 4).

Discussion

There are an abundance of studies regarding the stunning of (mostly younger) pigs with CO_2. The information also includes data from arterial and/or venous blood (Woodbury & Karler 1960; Forslid & Augustinsson 1988; Erhardt *et al* 1989; Martoft 2001; Machold *et al* 2003a,b).

Data regarding the effect of CO_2 narcosis under normal conditions and during regular slaughter work are, to our knowledge, lacking. This study should therefore provide information as to the extent to which the values of blood-gas partial pressures and acid-base parameters reveal diagnostic evidence of the depth of narcosis in pigs designated for bleeding.

Diagnostic relationship between the reflex response and parameters in slaughter blood

The depth of narcosis can be determined by the response to certain reflexes within the body, eg the corneal reflex (Cantieni 1977; Hertrampf & von Mickwitz 1979; Erhardt *et al* 1989; Troeger & Moje 2000; Remien 2001; Martoft *et al* 2002; Anonymous 2003). A lack of response to the corneal reflex implies stage III narcosis (Guedel scheme), including the absence of excitation and sensation of pain (von Brandis & Kilian 1931; Becker 2005).

Stunned pigs that do not show a corneal reflex can therefore be assumed to have reached stage III. At this stage, the bleeding of pigs should be pain- and stress-free. The evaluation of blood-gas partial pressures and acid-base parameters requires knowledge about the respective reference values. We have not found physiological data in the literature relating to the substrate 'slaughter blood'. The bleeding stitch presumably opens both arteries and veins proximal to the heart. The received slaughter blood can therefore be considered to be mixed arteriovenous blood. The total volume of blood (100%) is generally acknowledged to be

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subdivided into the 80% that occurs in the venous circuit and the 20% in the arterial circuit (von Engelhardt 2005). Our data therefore are more likely to closely represent values of venous as opposed to arterial blood.

Reference values in Figures 1–4 are demonstrated according to the findings of Hannon *et al* (1990), Kaplan *et al* (2006), Kaneko *et al* (2008) and Reinhold *et al* (2010).

Excessive CO_2 intake induces two different reactions in the pig. First, in unchanged overall air pressure, high CO_2 content blocks the percentage of other breathable gases, including that of oxygen, in the air. According to Remien (2001), a percentage of 90% CO_2 in the CO_2 chamber (the conditions in our study) leads to a decrease to less than 5% by volume of oxygen in the breathable air. For pigs narcotised in this manner, hypoxaemia (decreased pO_2 in the blood) and hypoxia (decreased pO_2 in the soft tissues, eg the nervous system) can accordingly be expected.

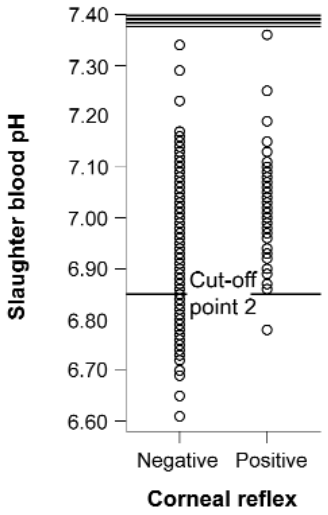
In this study, the majority of narcotised pigs showed decreased pO_2 at the time of blood collection (Figure 1). In some animals, however, normo- or hyperoxaemia were also detected. One reason for this might be the short-term breathing depression or even apnoea in the CO_2 chamber; the animals thus inspired the CO_2-enriched air to a low extent. A reflexive bronchial constriction is well known to serve as a protective body mechanism when irritant gases or fluids are present, eg NH_3 or acetic acid (Gros 2005). Pigs were exposed to normal air (O_2 = 20.9% by volume; CO_2 = 0.03% by volume) over a variable time range after the release from the CO_2 chamber and prior to bleeding (see Table 1). The normo- to hyperoxaemic findings can therefore be explained by the progressing alveolar normo- or hyperventilation of the reversibly narcotised pigs. While the pigs were suspended, we found a degree of perfunctory arrhythmic respiration in stunned pigs. However, these external observations are insufficient to deduce whether alveolar ventilation and actual gas exchange has occurred in animals' lungs.

The diagnostic quality of the pO_2 determined from slaughter blood in order to detect either a sufficient or insufficient depth of narcosis, ie positive or negative reflex response, indicated a limit of 3.1 kPa with reasonable sensitivity and specificity, viz 72 and 59%, respectively (Table 3). Nevertheless, at a threshold of ≤ 1.6 kPa, none of the pigs had a positive reflex response and, therefore, a sufficient narcosis depth had been reached (see also Table 2).

The excessive amount of CO_2 is absorbed by simple diffusion and passed through biological membranes to all body tissues, leading to systemic hypercapnia (increased pCO_2). The enzyme, carbonic anhydrase, which is present in many body cells, causes the physically diffused CO_2 to become hydrated within seconds and results in respiratory acidosis through the dissociated carbonic acid (increased H^+ ions or decreased pH, respectively). The extreme hypercapnia (Figure 2) and respiratory-induced acidaemia (Figure 3) found in the slaughter blood of stunned pigs confirms these previously stated assumptions.

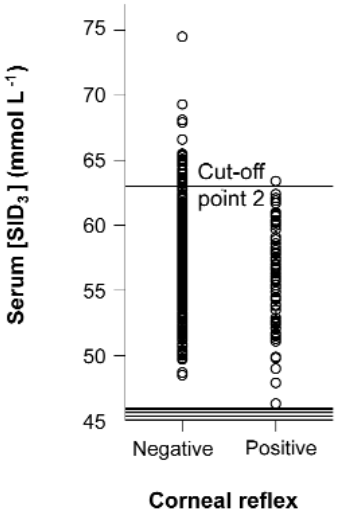
The diagnostic quality of the slaughter blood parameters pCO_2 and pH, with regard to the depth of narcosis in stunned pigs, is shown by threshold values of ≥ 20.1 kPa and a pH of ≤ 6.97; with a moderate sensitivity and specificity (Table 3). When threshold values for pCO_2 ≥ 23.0 kPa and pH ≤ 6.85 were implemented, positive responses to the corneal reflex occurred rarely or not at all in the pigs. Based on these cut-off points, the slaughter pigs were in a sufficiently deep narcosis.

Figure 3



Scattergram for pH values in slaughter blood (mixed arteriovenous blood) and tested response to corneal reflex immediately before bleeding in pigs narcotised with CO_2 prior to slaughter (n = 598; negative = 530, positive = 68 animals). Normal values (striped area): $pH_{arteriovenous}$ = 7.38–7.53.

Figure 4



Scattergram for $[SID_3]$ values in slaughter blood (mixed arteriovenous blood) and tested response to corneal reflex immediately before bleeding in pigs narcotised with CO_2 prior to slaughter (n = 598; negative = 530, positive = 68 animals). Normal values (striped area): Serum $[SID_3]$ = 45 ± 2 mmol L^{-1}.

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Of particular interest is the biological importance of the increased serum [SID_3] (Figure 4) in the stunned pigs. Systemic respiratory acidosis is generally considered to be compensated for by non-respiratory, ie metabolic parameters of the acid-base status, eg serum [SID_3] (Hartmann & Berchtold 2009). Metabolic compensation mechanisms in the renal system are initiated via modulation of electrolyte excretion (active tubular reabsorption and/or secretion). At least a couple of hours, and, ideally, one-to-two days, are necessary to detect these activities in the living animal. Differing from this assumption, all narcotised pigs revealed consistently increased, ie alkaline-induced, [SID_3] values in their slaughter blood (Figure 4).

Pigs narcotised with CO_2 develop a distinct reflex-induced bradycardia (Martoft 2001; Berencsy 2005) with a secondary reduced perfusion of the kidneys. The increased serum [SID_3] levels are unlikely to be correlated with the decreased perfusion of the kidney. Indeed, the electrolyte imbalances caused by the CO_2 excess (Bennett & Hayward 1967; Kanaan *et al* 2007) are potentially the reason for the fast alkaline reaction of serum [SID_3]. The examined pigs revealed hyperkalaemia and hyperchloraemia, in particular (Table 3), resulting in increased serum [SID_3] (Figure 4; Hartmann & Berchtold 2009). To our knowledge, no existing details are available in the literature concerning the reaction of Stewart variables as related to the acid-base status in stunned pigs.

Physiological processes occurring in the pigs after exposure to an excess of CO_2

The increasing oxygen deficiency during CO_2 exposure may provoke hypoxic vasoconstriction, via the Euler-Liljestrand reflex, in the entire pulmonary area of the slaughter pigs. Consequently, the pulmonary gas exchange is impaired due to an imbalance in the ventilation/perfusion ratio.

Hypoxaemic hypoxia in animals tends to be compensated for, to a certain extent, by the release of erythrocytes (spleen contraction) and increased cardiovascular output (hyperperfusion). However, according to Martoft (2001), pigs develop distinct bradycardia within seconds of CO_2 anaesthesia, accompanied by a decreased heart rate from > 115 beats per min (basal value) to less than 20. This reaction, which occurs within seconds, is caused by a trigeminovagal reflex (Berencsy 2005). After excess CO_2 inhalation, a rapid and distinctive decrease in heart rate occurs through the activation of receptors in the naso-mucosal membranes. According to this, respiratory-generated hypoxia in soft tissues is aggravated by ischaemia. There is consensus in the literature that the induced hypercapnia, and to a greater extent, the intra- and extracellular respiratory acidosis in the pig, are caused by the anaesthetic effects of the intensively inhaled CO_2 (Bennett & Hayward 1967; Gu *et al* 2007).

According to various authors, eg Erhardt *et al* (1989), Raj (1999) and Martoft *et al* (2002), one might anticipate that a CO_2 exposure time of > 60 s and a concentration of 80–90% by volume would cause modifications in neurological activity. After a timeframe of 44 s, animals revealed an 'isoelectric' electroencephalogram, suggesting severely reduced central nervous system (CNS) activity, ie 'brain death'. Remaining in the CO_2 chamber resulted in biological death after approximately 7 min in most of the pigs (Raj 1999). An exposure time of 1–2 min to 90% CO_2 by volume therefore certainly provoked reversible unconsciousness in the pigs under study. Details of which processes finally cause narcosis after excessive CO_2 intake are not yet known. Neurological alterations have been described in the literature, eg hyperpolarisation of sensitive cell membranes with an intracellular depletion of sodium/potassium and overall reduced central nervous excitability (Woodbury & Karler 1960; Kowalchuk *et al* 1988; Martoft 2001; Martoft *et al* 2002). One can generally assume that mild-to-moderate increased pCO_2 levels (> 6 kPa up to < 15 kPa) have stimulatory effects, whereas intensively increased pCO_2 (> 15 kPa) has an inhibitory effect on body functions (von Brandis & Killian 1931; Woodbury & Karler 1960).

According to Bennett and Hayward (1967) and Young and Peracchia (2004), gaseous molecules can be absorbed by cell membranes and gap junctions and alter their permeability. Membranous transport activities consequently become compromised (Kanaan *et al* 2007) resulting in reversible 'ion blocks' (Bennett & Hayward 1967). These conditions may explain the muscle relaxation observed in animals narcotised with CO_2 (Kowalchuk *et al* 1988; Allen *et al* 2008). In rats with an experimentally induced pCO_2 of > 19.2 kPa, suppression of the postsynaptic response has been observed (Siesjö 1985).

In contrast, decreased cerebral blood flow or energy deficiency caused by nerve hypoxia or glial cells is not supposed to have a considerable pathogenic impact (Folbergrová *et al* 1972).

A fundamental reason for rejecting CO_2 as a stunning method is based upon attitude surveys and vocalisation identifying aversions against the use of CO_2 in pigs (Raj & Gregory 1995, 1996; Jaresch 2001; Machold *et al* 2003a,b). Central chemoreceptors that are located ventral to the respiratory centre in the medulla oblongata and are highly susceptible to CO_2, are overly stimulated by the abrupt intake of CO_2 (Kawai *et al* 2006). Reflex pathways from the spinal cord to the CNS could thereby alter the perception of consciousness in pigs (Richter 2008).

Conclusion

The stage of narcosis was determined through both the corneal reflex and the evaluation of blood-gas pressures and acid-base parameters in arteriovenous slaughter blood. After the assessment of suitable cut-off points, the continued recording of pH and/or pO_2 values in the slaughter blood via ion-sensitive electrodes could provide a reliable monitoring system to determine the depth of narcosis for both abattoir and animal protection authorities. Since the data have been obtained directly from pigs at the point of operational activity to ensure successful CO_2 stunning, the collected data are of high biological significance.

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