PHYSIOLOGY, ENDOCRINOLOGY, AND REPRODUCTION

Susceptibility of Duck and Turkey to Severe Hypercapnic Hypoxia

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ABSTRACT Large groups of poultry, including ducks and turkeys, are killed for disease control purposes with CO_2. In this study, we examined the physiological reaction of White Pekin ducks and turkeys to increasing CO_2 concentrations. Additionally, we examined the suitability of killing both species with increasing CO_2 concentrations. Blood gas values showed similar reaction patterns for both species: a strong increase in pCO_2 from approximately 40 to 200 mmHg, decreasing pO_2 and O_2 saturation, a decrease in pH from 7.4 to 6.7, and a strong shift in acid-base equilibrium (averaging 0 to –23). On the electroencephalogram, θ and δ waves occurred at 21 to 23% CO_2, and suppression to a near isoelectric electroencephalogram occurred between 41.8 and 43.4% CO_2 in inhaled air. Heartbeat declined from approximately 300 beats per min (bpm) at the start to 225 bpm at loss of posture to 150 bpm at 1 min before the heartbeat ceased. During the last phase of heart activity, an irregular rhythm and fibrillation were observed in addition to a decline in bpm. Blood gas values and electrophysiological data confirmed that ducks and turkeys lose consciousness before a level of 25% CO_2 in inhaled air is reached and that both ducks and turkeys die within 13 min in an environment of 45% CO_2 in inhaled air.

Key words: carbon dioxide, blood gas, electrophysiology, duck, turkey

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INTRODUCTION

Diseases such as avian influenza (**AI**) can be controlled by killing all birds on infected farms as well as on the surrounding and suspected farms. In The Netherlands in 2003, this resulted in the killing of >30 million farmed birds, which successfully stopped the disease (Stegeman et al., 2004). However, despite the killing of large numbers of poultry worldwide, AI infections have not been stopped. In The Netherlands, the majority of birds were killed by increasing CO_2 concentrations in their housings. Evaluation of the process raised questions concerning the suitability of such methods for all poultry species. Moreover, the differences in susceptibility of ducks and turkeys to increasing CO_2 concentrations in inhaled air are questionable.

During the AI epidemic, it seemed difficult to kill ducks and turkeys in their housings with increasing CO_2 concentrations. However, it remained uncertain whether ducks and turkeys are less susceptible to these high CO_2 concentrations, compared with broilers and laying hens, or whether different housing conditions are responsible for the differences in response. The suitability of CO_2 for killing ducks is questioned based on the assumption that diving birds, including ducks, are less susceptible to asphyxia and hypoxia (Powell et al., 2004) and that they possess physiological mechanisms that enable them to withstand hypercapnia (Hawkins, 2001). However, White Peking ducks belong to the so-called dabblers, which do not dive (Belrose, 1981).

Experiments with broilers (Gerritzen et al., 2004) resulted in <0.05% survivors at concentrations of 35% CO_2 in inhaled air. Observations in practice during whole-house gassing of broilers and laying hens (Gerritzen et al., in press) made it clear that 40% CO_2 in inhaled air is sufficient to kill group-housed broilers and laying hens. Increasing CO_2 concentrations, and therefore decreasing O_2 concentrations, in inhaled air led to a hypercapnic-hypoxic state. Increasing pCO_2 levels led first to dyspnea followed by increasing respiration rate, but at high pCO_2 levels (>150 mmHg), CO_2 causes respiratory depression, resulting in a vicious circle of events that eventually results in death (Guyton and Hall, 2000). In experiments with pigs, Martoft et al. (2003) found that a decrease of arterial pH results in a decrease in pH of the cerebral fluid.

High levels of CO_2 led to the occurrence of high amplitude, low frequency activity and θ and δ waves (both indicative of unconsciousness) in broilers and hens (Raj et al., 1992, 1998; Gerritzen et al., 2004). In general, effects of hypercapnic and hypoxic conditions on the physiological state of ducks and other birds are well described for suboptimal situations, such as during diving and at high altitude (Jones and Holeton, 1972; Butler and Taylor, 1983; Shimizu and Jones, 1987; Borg et al., 2004). Blood gas

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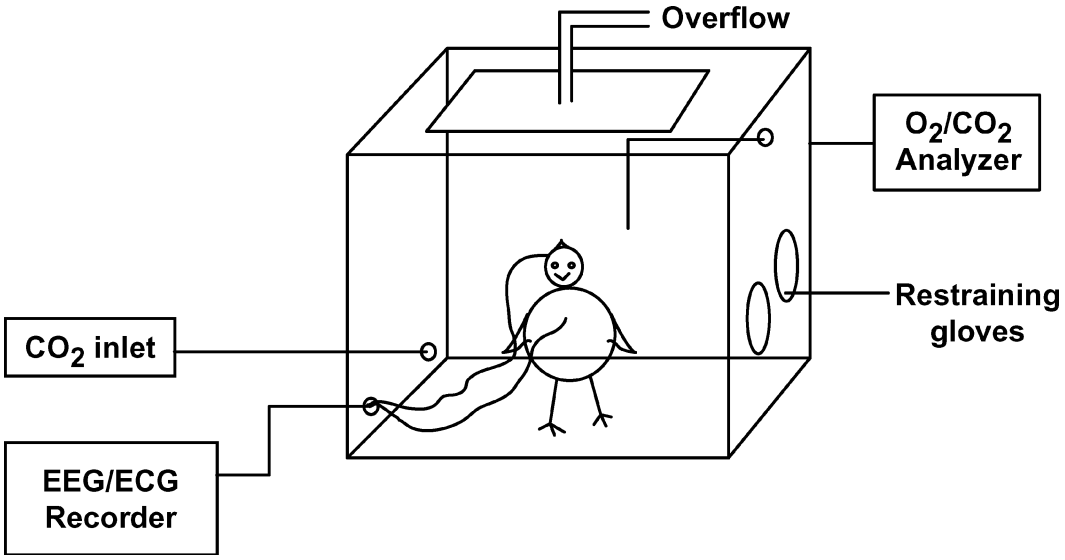

Figure 1. Schematic overview of the test situation. EEG = electroencephalogram; ECG = electrocardiogram.

values are indicative of the ability to react to hypercapnic and hypoxic situations. However, not much is known concerning the effects on blood gases because of extreme hypercapnic-hypoxic situations encountered during killing with high CO_2 levels.

The aim of this study was to provide physiological information to establish unconsciousness and death in ducks and turkeys and, thus, determine the suitability of increasing CO_2 concentrations for killing ducks and turkeys. In this paper, we present blood gas values and electrophysiological data, measured to provide information concerning the suitability of increasing CO_2 concentrations, for killing ducks and turkey.

MATERIALS AND METHODS

Animals

At the poultry facilities of the Animal Sciences Group (Lelystad, The Netherlands), twenty-five 2-wk-old White Pekin ducks (*Anas platyrhynchos*) and twenty-five 3-wk-old turkeys (*Meleagris gallopavo*) were raised under conditions similar to those found in the commercial poultry industry. All animals were housed on litter and had ad libitum access to feed and water. Experiments started when the animals were 6 to 7 wk of age. Ethical aspects of the experiment were judged and approved by the animal ethical committee of the institute.

Experimental Design

Euthanasia experiments were carried out with individual animals in a Perspex box (i.d. = 0.8 × 0.8 × 0.8; Figure 1). The test box was fitted with a 1-cm diameter CO_2 inlet located 5 cm above the base of the box. A CO_2 measuring tube was placed in the center of the box at sitting head height of the animals, and an overflow valve was situated at the top of the box. One of the Perspex sides of the test box was equipped with rubber gloves. Carbon dioxide was injected from a 100% CO_2 source at a flow of 14 L/min until 40% CO_2 in inhaled air was reached at the measuring point. The birds remained in this CO_2 concentration until they died. Death of the animals was judged visually based on behavioral and electrophysiological observations (i.e., absence of brain activity and heartbeat).

Both groups of animals were split into 2 groups: 1) a group of 15 animals equipped with electrodes and blood vessel catheters and 2) a group of 10 animals that was not operated on before the trials and were allowed to move freely in the test box during the trials. The operated animals were restrained manually during the trials to prevent interference with the electrodes and to minimize disturbance of the signal caused by bird movement. Therefore, free-moving animals were used as the reference group to assess loss of posture, the behavioral indicator for loss of consciousness.

Surgical Techniques

Fifteen ducks and 15 turkeys were equipped with brain electrodes for electroencephalogram (**EEG**) registration, electrocardiogram (**ECG**) electrodes for heartbeat registration, and a blood collection catheter in the wing vein (Vena cutanea ulnaris). The EEG electrodes and the blood vessel catheter were placed 24 h before the onset of the experiment.

Light narcosis was induced using inhalation anesthesia with 4% isoflurane in 200 mL of N_2O/min and 400 mL of O_2/min until the eyes closed; this was continued at 0.8% isoflurane. The duration of the narcosis varied from 15 to 25 min. Placement of the EEG electrodes was achieved through a 2-cm incision parallel to the comb and removal of the periost. Two silver electrodes (Engelhard-CLAL, Carteret, NJ; 55% silver, 21% copper, and 24% zinc) of 10 mm in length and a diameter of 1 mm were punctured through the skull on the line ear to ear, 0.5

cm left and right of the sagital line. The electrodes were attached to the skull and isolated from each other with dental cement. Electrode wires were tunneled under the skin from the head to the back and fixated on the back between the base of the wings by a double ligature and tape. The skin of the skull was closed by 3 single sutures (vycril 2-0).

A blood collection cannula (type: braunule; 40 mm × 18-gauge was placed in the right or left wing vein by directly puncturing the vein through the skin. The catheter was brought 4 cm into the vein, filled with a 5% heparin solution, and closed with a stopcock. The outer part of the catheter was fixed at the wing by 3 single crossing sutures (vycril 2-0).

Isoflurane and N_2O were stopped, and extra O_2 was administered until the chickens opened their eyes and could maintain a sitting position. Immediately after the operation, animals were housed individually in the same room as the other animals and allowed 24 h to recover.

For the registration of heart rate immediately prior to the trial, 2 stainless-steel electrodes (4 cm long and 0.5 mm in diameter) were punctured through the skin and placed subcutaneously at the left and right side of the breast, directly under the wing base. To avoid disturbance to the EEG and ECG signal, a stainless-steel, earth-connecting electrode was placed subcutaneously on the back. The electrodes were attached to the birds' body with a tape bandage.

Blood Parameters

Blood samples of 1 mL were collected anaerobically into a heparinized, 2-mL polyethylene syringe immediately prior to CO_2 inlet, just after loss of posture (i.e., at the moment of loss of consciousness) and directly after the animals died (i.e., after all heartbeat stopped). The samples were immediately placed on melting ice and analyzed within 15 min. Blood samples were mixed, and a 0.2-mL sample was injected into a mobile acid-base laboratory (ABL 605, Radiometer Nederland BV, Zoetermeer, The Netherlands) and analyzed for pCO_2, pO_2, O_2 saturation (**O_2 sat**), pH, acid-base equilibrium (**ABE**), HCO_3^-, glucose, and lactate.

Electrophysiological Measurements

The electrodes were connected with the measurement and recording device by isolated and shielded wires. The microvoltage signals of the brain and heart were amplified using a bioamplifier (model BMA-931, CWE, Inc., Ardmore, PA) and continuously measured and recorded using Windaq computer software (DATAQ Instruments, Akron, OH). The recorded files were analyzed later. The EEG signals were analyzed for changes in frequency and amplitude. Changes in frequency, more specifically, the suppression of α (8 to 13 Hz) and β (>13 Hz) waves and the occurrence of θ (4 to 8 Hz) and δ (<4 Hz) waves are indicative of loss of consciousness. Suppression of θ and δ waves will lead to an irreversible isoelectric EEG. The ECG signals were analyzed for heart rate at the start of the gas flow, at loss of posture, and during the last minute of heart activity.

Statistical Analyses

The effect of restraining the animals was analyzed using ANOVA:

$$Yij = \mu + Si + Rj + SRij$$

where Yij is the response variable, μ is the general mean, Si (i = 1, 2) is the main factorial effect of species, Rj (j = 1, 2) is the factorial effect of amount of restriction (yes or no), and SRij is the interaction effect of species and restriction. To analyze electrophysiological and blood gas data, the model was reduced to $Y = \mu + S$.

The assumption of normality of the model was visually tested using plots of between- and within-session residuals. Factorial effects and differences between means were declared significant when their probability levels, based on the between-sessions residual error only, were <0.05.

All analyses were carried out using GENSTAT 7 software.

RESULTS

Loss of posture for free-moving ducks and turkeys occurred at a significantly lower CO_2 concentration than for restrained ducks and turkeys. These CO_2 concentrations averaged 22.7 and 26.9% CO_2 for free-moving and restrained ducks, respectively, and 19.2 and 23.4% for free-moving and restrained turkeys, respectively.

Blood Parameters

In Figure 2, blood gas values are presented as ANOVA means ± standard errors of the pairwise differences.

Blood Gas Values. At normal atmospheric CO_2 concentrations in inhaled air, the pCO_2 in venous blood was lower (P = 0.001) in ducks (38.0 mmHg) than in turkeys (56.9 mmHg).

Levels of pCO_2 measured directly after loss of posture did not differ significantly between ducks (89.0 mmHg) and turkeys (79.3 mmHg). Directly after death, pCO_2 increased to 187.1 mmHg for ducks and to 224.0 mmHg for turkeys; pO_2 started at the same level for both ducks (54.6 mmHg) and turkeys (47.4 mmHg). The pO_2 was significantly lower directly after loss of posture ($P = 0.047$) and death ($P = 0.029$) for ducks (41.4 and 19.3 mmHg, respectively) than for turkeys (51.6 and 36.3 mmHg, respectively). This resulted in a stronger decline in pO_2 for ducks than for turkeys. The decline in O_2 sat followed the same course as the decrease in pO_2, resulting in a significantly lower O_2 sat at loss of posture ($P = 0.022$) and death ($P = 0.0\ 12$) for ducks (52.3 and 7.2%, respectively) vs. turkeys (73.9 and 31.3%, respectively).

Acid-Base Status. At the start of the CO_2 inlet, there was no significant difference in pH between ducks (7.38)

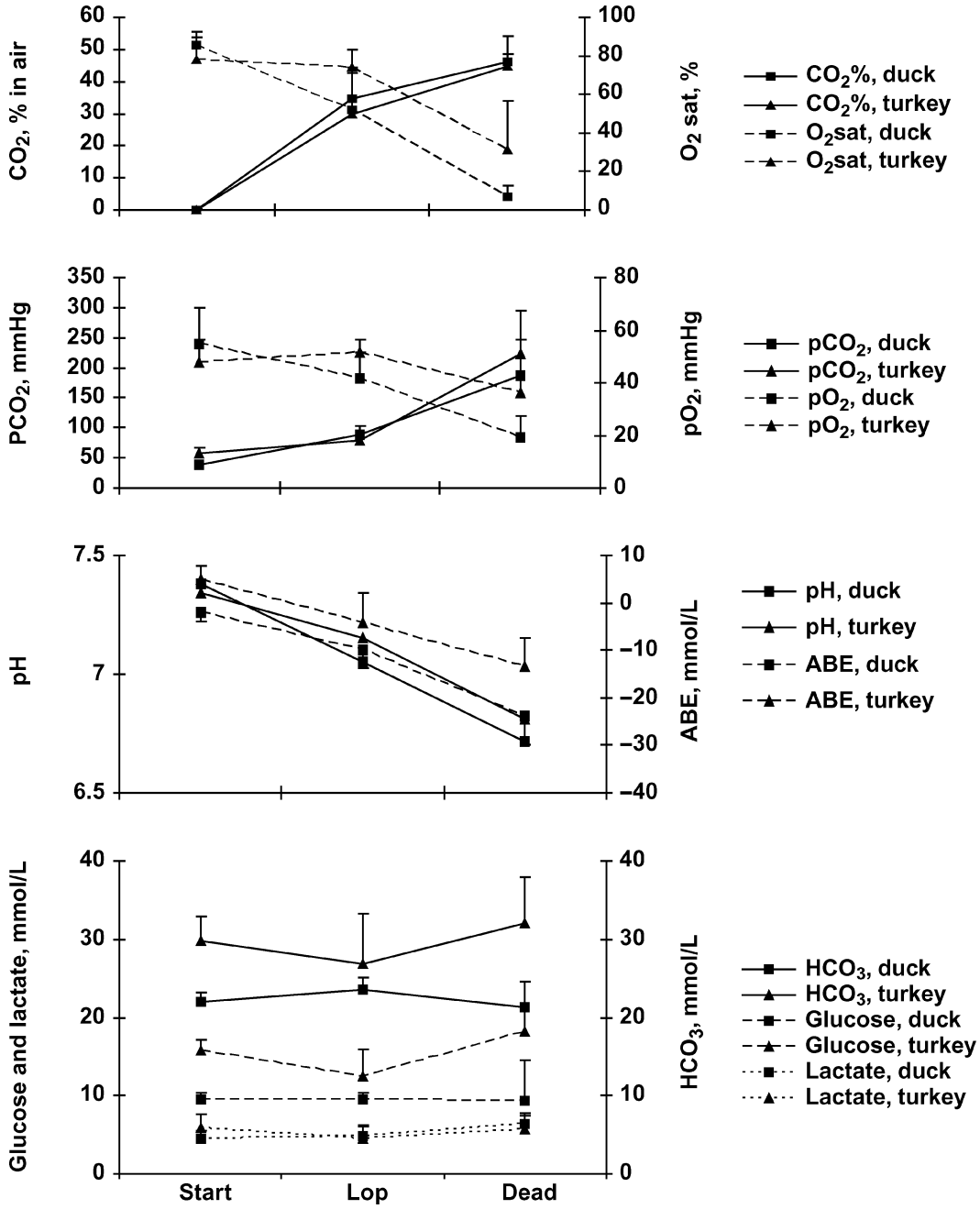

Figure 2. Venous blood gas values of ducks and turkeys measured before starting the CO_2 flow (Start), directly after loss of posture (LOP = loss of consciousness), and directly after the birds died (Dead). O_2 sat = O_2 saturation; ABE = acid-base equilibrium.

and turkeys (7.34). At loss of posture, a small but significant difference in pH was measured between ducks (7.05) and turkeys (7.16). The pH difference, although not significant, was also measured directly after death, 6.71 for ducks and 6.81 for turkeys. Levels of HCO_3^- were stable from start to death in both species. However, HCO_3^- levels were significantly lower for ducks (22.4 mmol/L) than for turkeys (29.5 mmol/L). Acidosis was clearly expressed in the ABE; both species showed a strong decline in ABE. At the start, ABE was significantly lower for ducks (–2.1 mmol/L) than for turkeys (4.9 mmol/L). However, for both species, this was close to the neutral value of 0. At loss of posture, ABE decreased for both species by approximately 7 to 8 mmol/L. Between loss of posture and death, ABE decreased more strongly for ducks (from –9.9 to –23.4 mmol/L) than for turkeys (from –4.4 to –13.4 mmol/L).

Metabolic Parameters. Glucose levels remained stable in ducks from start to loss of posture and death (respectively, 9.5, 9.5, and 9.3 mmol/L). However, glucose levels fluctuated in turkeys from 15.8 at the start, to 12.4 at loss of posture, to 18.2 directly after death, resulting in a significant difference ($P < 0.02$) between ducks and turkeys at all 3 moments. Lactate levels did not differ between species and remained stable from the start until death (approximately 5.3 for ducks and 5.4 for turkeys).

Table 1. Carbon dioxide concentration (%) in inhaled air at the time of changes in brain activity and at loss of posture of birds equipped with brain electrodes. Data presented are ANOVA means

Parameter	Duck (n = 5)	Turkey (n = 5)	P (F)[1]	SED[2]
θ/δ waves	21.0[3]	23.3	0.589	4.0
Strongly suppressed	43.4	41.8	0.287	1.4
Loss of posture	25.3	25.5	0.963	3.5

[1]ANOVA significance level of the *F*-test; df of the residuals = 1, 21.

[2]SED = standard error of the pairwise differences.

[3]Means within a row are significantly different at $P < 0.05$ using student's *t*-test.

Electrophysiology—EEG. Theta and δ waves (Table 1; Figure 3) occurred at the same concentration of CO_2 in inhaled air for both ducks (21%) and turkeys (23%). For both species, θ and δ waves occurred before loss of posture, at a level of 25% CO_2 for ducks and at 26% CO_2 for turkeys. Strong suppression of the EEG to near isoelectric occurred at similar CO_2 concentrations for ducks (43.4%) and turkeys (41.8%).

Electrophysiology—ECG. At the start of the trials, there were no significant differences in heart rate (Table 2; Figure 3) between ducks [309 beats/min (**bpm**)] and turkeys (296 bpm). At loss of posture, no significant difference in heartbeat was observed between ducks (206 bpm) and turkeys (233 bpm). However, the decrease in heart rate between start and loss of posture was larger for ducks than for turkeys. One minute before death, ducks still had a lower, although not significant, heart rate (136 bpm) than turkeys (164 bpm). The duration until all heartbeat ceased did not differ significantly between turkeys (8.8 min) and ducks (9.8 min). During bradycardia and shortly before cardiac arrest, fibrillation and arrhythmic heart rate occurred in all animals.

DISCUSSION

Blood gas values and electrophysiological parameters were measured to assess the suitability of increases in CO_2 concentrations for killing White Pekin ducks and turkeys. During emergency killing, the most important criterion is that all animals are killed by the initial method.

Table 2. Heart rates per min before starting the CO_2 inlet [beats per min (bpm) start], at loss of posture (bpm at lop), 1 min before cessation of all heartbeat (bpm before death), and absence of heart rate (min after starting the gas inlet) of birds equipped with breast electrodes. Data presented are ANOVA means

Parameter	Duck (n = 5)	Turkey (n = 5)	P (F)[1]	SED[2]
bpm start	309[3]	297	0.579	21
bpm at lop	206	233	0.346	26
bpm before death	136	164	0.267	24
No heartbeat (min)	9.8	8.8	0.539	1.6

[1]ANOVA significance level of the F-test; df of the residuals = 1, 21.

[2]SED = standard error of the pairwise differences.

[3]Means within a row are significantly different at $P < 0.05$ using student's *t*-test.

For broilers, CO_2 concentrations of 35% in inhaled air result in <0.05% survivors (Gerritzen et al., 2004). Observations during the AI epidemic in 2003 (Gerritzen et al., in press) made it clear that 40% CO_2 in inhaled air is sufficient to kill all broilers and laying hens in their housings. In a small-scale pilot experiment (unpublished) with 4 turkeys and 4 Pekin ducks, loss of consciousness and death occurred at the same level of CO_2 in inhaled air as for broilers. During the present experiment, CO_2 concentrations in inhaled air increased from 0 to 45% within 10 min, introducing a progressive hypercapnic-hypoxic situation. The hypercapnic-hypoxic state was maintained until all signs of life disappeared (i.e., no breathing and an isoelectric EEG and ECG). Until now, it was questionable whether these conditions would be extreme enough to kill other poultry species (i.e., ducks and turkeys). Hawkins (2001) stated that ducks and diving birds possess physiological mechanisms enabling them to withstand hypercapnia and, therefore, prolong death.

In the present study, ducks and turkeys became unconscious at 20 to 25% CO_2 in the breathing air. Loss of consciousness was established from the EEG, based on loss of posture. However, the animals were manually restrained, and the reaction time of the restraining person presumably had an effect on the data. Also, the resistance of the animals to the restraining method may be responsible for the delay in loss of posture and, thus, for a delay in loss of consciousness. Suppression of the α and β waves, together with the occurrence of θ and δ waves, are typical of loss of consciousness (Mattson et al., 1972; Forslid, 1987; Raj et al., 1998). For both ducks and turkeys, θ and δ waves occurred directly after loss of posture. The changes in EEG signal and the complete loss of posture, as seen in this experiment, agree with results found in broilers (Raj et al., 1998; Gerritzen et al., 2004) and turkeys (Raj and Gregory, 1993). At the point of occurrence of θ and δ waves in the present experiment, pCO_2 levels in venous blood increased to 80 to 110 mmHg. In humans, when alveolar pCO_2 rises to 80 to 100 mmHg, the person becomes lethargic and semicomatose (Guyton and Hall, 2000). Shortly after loss of consciousness, pCO_2 values in venous blood of pigs increased to 16.6 kPa (±124 mmHg), and pH fell from 7.4 to 7.0 when inhaling 80% CO_2 (Forslid and Augustinsson, 1988). Furthermore, increasing pCO_2 levels parallels the decrease in blood pH, which results in a reduction of pH in cerebral fluid (Martoft et al., 2003). Acidosis of the cerebrospinal fluid caused by increasing CO_2 levels will lead to unconsciousness (Woodbury and Karler, 1960; Eisele et al., 1967; Kohler et al., 1999). The normal pH of cerebrospinal fluid is 7.4, and a state of analgesia and anesthesia is induced at 7.1 and 6.8, respectively (Eisele et al., 1967). The progressive increase in CO_2 concentration resulted in venous pCO_2 tension over 200 mmHg with pH decreasing to 6.6. Levels of pCO_2 of 120 to 150 mmHg in humans lead to anesthesia and death (Guyton and Hall, 2000). Increases in CO_2 tension and decreasing pH are, in the first place, controlled by increasing respiration rate. Increased ventilation washes CO_2 out and functions as a mechanism for the removal of acid

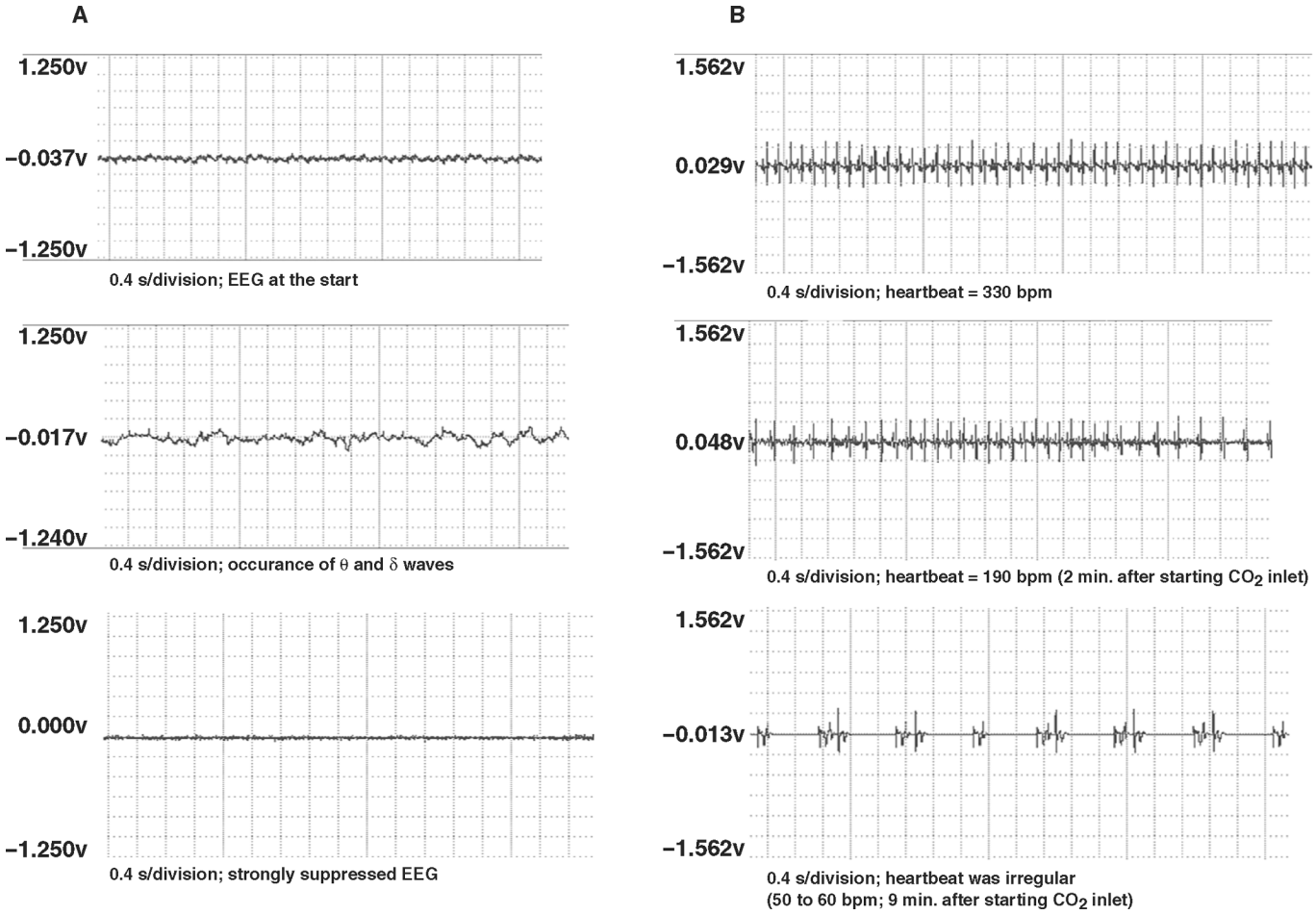

Figure 3. A) Example of essential parts of an electroencephalogram (EEG) of a duck at the start of a trial, at loss of consciousness, and shortly before death. B) Example of essential parts of an electrocardiogram of a duck at the start of trial, at loss of consciousness, and shortly before death. bpm = beats per minute.

from the blood. As a result, serum pH decreases, pCO_2 decreases, and serum bicarbonate will decrease (Chandrasoma and Taylor, 1995). However, because of the high CO_2 level in inhaled air and the increased ventilation, CO_2 tension in the blood will increase dramatically. Excessive CO_2 tension will depress respiration rather than stimulate it, leading to a vicious circle of events ending in respiratory death (Guyton and Hall, 2000). In experiments with pigs, arterial pH decreased from 7.5 to 6.8 during a 1-min exposure to 90% CO_2 and subsequently returned to normal levels after CO_2 inhalation stopped (Martoft, 2001). In the present study, the pH of venous blood decreased from 7.4 at the start to 6.6 measured immediately after death. Acidosis is clearly visible in the strongly decreasing ABE (–18 to 20 mmol/L). In addition to respiratory compensation, ABE is controlled by H^+ excretion from the kidneys, causing retention of HCO_3^-. In the present study, HCO_3^- remained almost constant, which, following the Henderson-Hasselbach equation, together with increasing pCO_2 and increasing H^+ concentrations, leads to acidosis. Changes in plasma pH have a marked impact on enzyme reactions associated with energy production, changes in membrane permeability, and electrolyte transfer.

Parallel to the high pCO_2 levels and the acidosis, the O_2 sat, and therefore the pO_2 of the blood, decreased, leading to hypoxia. Effects of hypoxia on the central nervous system are manifest as dizziness, fainting, and lethargy. Normally, hypoxia is compensated by increasing heartbeat and an increased stroke volume of the heart. Increased heart rate as a response to a reduction in O_2 tension in the blood was also observed in ducks (Jones and Holeton, 1972). In experiments with diving ducks (Borg et al., 2004), bradycardia was seen only following extreme hypoxia. In the present study, the level of hypoxia observed at the time of loss of consciousness is suspected as more likely to have induced a tachycardia than the observed bradycardia. It has to be considered that heartbeat, especially at the start of a trial, is most likely increased by human restraining (Heise, 1989). After loss of consciousness, hypoxia increased further, resulting in bradycardia and arrhythmic heartbeat, ending in heart failure.

In the present study, all animals died within 13 min in an atmosphere where CO_2 was gradually increased from 0 to 44%. Because differences in blood gas values and electrophysiological data were only small between ducks and turkeys, it is not considered necessary to stipulate

different criteria for these species. Furthermore, the physiological ability of ducks to respond at hypercapnia and hypoxia, as is seen under suboptimal conditions during diving or at high altitudes (Andersen and Lövö, 1964; Powell et al., 2004), was not observed in this experiment. The White Pekin duck is the domestic variety of the mallard. Mallards belong to the group of dabbling ducks that usually find their feed in shallow water and on land and usually do not dive (Belrose, 1981). Therefore, it can be questioned whether domestic White Pekin ducks have the ability to respond to hypoxia or hypercapnia as do diving birds. Response of the same birds to their pathophysiological state was not always as expected. However, all described factors (i.e., the progressive hypercapnia as well as severe hypoxia and the acidosis) are able to induce a pathological state, causing death. However, the conditions, 40% CO_2 maintained for 30 min, as stated earlier for chickens (Gerritzen et al., 2004), may be critical for ducks and turkeys. Therefore, to kill all farmed poultry species, it is recommended that a minimal concentration of 45% CO_2 in inhalation air be maintained for 30 min.

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