Mass depopulation of laying hens in whole barns with liquid carbon dioxide: Evaluation of welfare impact

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ABSTRACT Appropriate emergency disaster preparedness is a key priority for agricultural agencies to allow effective response to serious avian disease outbreaks. There is a need to develop rapid, humane, and safe depopulation techniques for poultry that are widely applicable across a range of farm settings. Whole barn depopulation with carbon dioxide (CO_2) has been investigated as a humane and efficient means of killing large numbers of birds in the event of a reportable disease outbreak. It has also been considered as a method for depopulating barns containing end-of-lay hens, particularly when there is limited local slaughter and rendering capacity. Determining the best method of humanely killing large flocks of birds remains problematic and is being investigated by a coordinated international effort. While whole barn depopulation using CO_2 inhalation has been explored, physiologic responses of chickens have not been characterized in field settings and assessment of animal welfare is hampered without this information. In this study, 12 cull laying hens were surgically instrumented with telemetry transmitters to record electroencephalographs, electrocardiographs, body temperature, and activity during 2 large-scale field CO_2 euthanasia trials of end-of-lay hens. The day following surgery, instrumented hens were placed in barns with other birds, barns were sealed, and animals were killed by CO_2 inhalation delivered via a specially designed liquid CO_2 manifold. Instrumented birds were monitored by infrared thermography, and ambient temperature, CO_2, and O_2 concentrations were recorded. Results from these studies indicate that instrumented hens lost consciousness within 2 min of CO_2 levels reaching 18 to 20%. Mild to moderate head shaking, gasping, and 1 to 2 clonic muscle contractions were noted in hens before unconsciousness; however, brain death followed rapidly (<5 min). Evaluation of welfare costs and benefits suggest clear advantages over catching and transporting cull hens for slaughter. The financial costs with this method are greater, however, than those estimated for traditional slaughter techniques. Results of these studies are being used to develop national protocols for whole barn depopulation of hens by CO_2 inhalation.

Key words: mass depopulation, animal welfare, liquid carbon dioxide, whole barn, telemetry

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INTRODUCTION

Recent global outbreaks of avian influenza and exotic Newcastle disease in domestic and wild avian species have highlighted the need for appropriate agricultural biosecurity practices and emergency disaster preparedness (Gilpen et al., 2009; Honhold et al., 2011). Both diseases are caused by viruses that can infect a broad range of avian species and are spread via direct contact by infected birds and indirectly through contact with contaminated equipment and fomites. Because of this, once the diseases are identified, the goal of the Canadian Food Inspection Agency and other poultry industry groups is to eliminate all potentially infectious birds from quarantined farms and wildlife centers as rapidly as possible to minimize disease spread. This requires a failsafe, readily available, humane procedure for mass depopulation of birds.

In addition to disease outbreaks, there is limited slaughter capacity for end-of-lay (spent) hens in Canada. Currently, spent layers may be transported for significant distances from the farms of origin to facilities for slaughter. Some provinces do not have plants with suitable slaughter facilities, highlighting the urgent necessity for development of alternative techniques for humane depopulation of poultry. Development of humane and effective on-farm depopulation practices

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would eliminate potential handling, fasting, and transportation stress experienced by these animals, which are often markedly calcium-deficient and susceptible to fractures (Gregory and Wilkins, 1989; Knowles and Wilkins, 1998). Further, any disease outbreak or labor dispute that eliminated or reduced transportation or slaughter capacity would rapidly create a backlog of spent hens, potentially involving hundreds of thousands of birds across Canada.

By definition, euthanasia refers to the humane killing of an animal accomplished by a method that produces rapid unconsciousness or anesthesia and subsequent death without evidence of pain or distress (AVMA, 2007). Determining the best method of euthanizing large flocks of birds remains problematic and is currently being investigated by a coordinated international effort. Because of animal welfare, biosecurity, and occupational health and safety concerns, the selected method must be relatively rapid, humane, safe for the operators, readily available nationally, and easy to use. Ideally, the technique should be transferable to other avian species and should be broadly applicable to a range of housing conditions and circumstances. The available methods include barbiturate injection, inhalant anesthesia, carbon dioxide inhalation, carbon monoxide inhalation, mixtures of these gases with other inert gases, and gas-filled expansion foam (Raj, 2008). For this study, we chose to investigate a method involving carbon dioxide gas inhalation, because of its relative safety, ease of use, and ready commercial access to large volumes of liquid CO_2. We also selected an experimental approach to whole barn depopulation that would minimize the need to physically handle large numbers of live birds, thus reducing the potential negative effect of handling on birds, as well as reducing the number of personnel required and those potentially adversely affected by the procedure. Whole barn gassing requires sealing of the building, which may not be possible for all types of layer barns, but which is well-suited to typical layer barn construction in Canada and colder climates.

Carbon dioxide is a gas at standard room temperature and pressure and is colorless and odorless at low concentrations, with a sharp acidic odor perceived by humans at higher concentrations. It is an acidic oxide, with a pKa of 6.35, and it freely interconverts to carbonic acid (H_2CO_3) in aqueous environments. Under terrestrial conditions it exists only in the solid or gaseous state, and liquid CO_2 is formed by compression and immediately converts to a 2-phase solid and vapor state at atmospheric pressure, with a temperature of approximately −78°C (Praxair, 2007). Carbon dioxide has been used for euthanasia of a range of animal species for many years and is thought to induce analgesia and anesthesia at low doses in birds via induction of hypercapnic hypoxia (Lambooij et al., 1998). Carbon dioxide inhalation is considered an acceptable technique for poultry euthanasia according to the AVMA (2007) Guidelines on Euthanasia, and is also used for stunning at slaughter. In addition, the OIE (World Organization for Animal Health) has recently concluded that the agent of choice for depopulating a large number of birds is CO_2 (Galvin et al., 2005). A concentration of 40% CO_2 by volume in air has been reported to be sufficient to reliably kill broilers and laying hens (Gerritzen et al., 2006a,b).

Use of carbon dioxide gas inhalation for euthanasia is not without controversy. In mammalian species, there are concerns that exposure to CO_2 gas may be aversive, inducing pain and distress, and alternative methods are encouraged (Eisele et al., 1967; Conlee et al., 2005; CCAC, 2010). Further, in a whole barn setting, changes in ambient temperature before the birds have lost consciousness are of concern due to the very low delivery temperature of liquid CO_2 (Raj et al., 2006). Mass euthanasia techniques for poultry using carbon dioxide have been reported recently; however, the physiologic responses of chickens during this procedure have not been well studied, particularly in farm settings (Savory and Kostal, 1997). Thus, the objectives of this study were to optimize CO_2 delivery conditions in an empty barn pilot study, and then to investigate the physiologic effects of whole barn CO_2 gassing on end-of-lay hens held under both caged versus free-range conditions, and to compare the findings with economic and welfare cost estimates associated with typical transportation and slaughter practices. Together with representatives from the Canadian Food Inspection Agency (CFIA), Canadian Veterinary Medical Association (CVMA), Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Alberta Agricultural Research Institute, and animal welfare and poultry industry representatives, our efforts are directed toward determining practical options to depopulate avian species in a safe and humane manner.

MATERIALS AND METHODS

Empty Barn Pilot Study of Environmental Conditions

A pilot study was conducted using the second floor of an empty broiler breeder barn with characteristics as described (Table 1). A central floor scratch area was bordered by 2 rows of nesting boxes elevated 0.15 m on raised decks along both long sides of the building. Following a 24-min period of gas delivery, the barn remained sealed to evaluate maintenance and changes in gas concentrations over time.

Birds and Experimental Conditions

Experiment 1. This study was conducted with a flock of 24,000 caged layer White Leghorn chickens (*Gallus domesticus*). Cull hens were 72 wk of age at the time of study and had been in production for the preceding 52 wk. Eight hens weighing between 1.62 and 1.98 kg were selected for telemetry instrumentation. Birds were

Table 1. Specifications of barns and CO_2 use for whole barn liquid CO_2 trials

Trial	No. of hens	No. of manifolds	Barn length (m)	Barn width (m)	Ceiling height (m)	Barn volume (m^3)	Amount of CO_2 required (kg)	Trial duration (min)
Pilot study	0	2	58.5	12.5	2.6	1,827	3,910	24
Experiment 1	24,000	4	83.5	12.2	3	3,102	5,185	14
Experiment 2	13,100	4	140.2	12.2	3.7	6,249	11,975	32

housed in suspended galvanized metal cages (5–6 hens per cage) with front feeders and sloped cage floors for automated egg collection at the cage front. Cages were arranged in 5 rows with 4 tiers of cages per row (approximately 6,200 cages) within a steel-clad, insulated stud wall barn with a poured concrete floor, in-floor piped water heating, and a partial concrete side wall (Table 1). The barn was equipped with a belt manure system under the cages, and manure was moved by auger to an adjacent manure storage shed. The barn was mechanically ventilated with overhead air inlets and exhaust fans located on exterior walls. Ventilation fans were situated just above ground level. The outside air temperature was 9°C for this trial.

Experiment 2. The second study was conducted with a flock of 13,100 free-range ISA Browns. Cull hens from this barn were also 72 wk of age at the time of study and had been in production for one year. Four hens selected for telemetry instrumentation weighed approximately 1.7 kg each. Birds were loose-housed on slatted flooring with a manure belt underneath. An automated nest system ran the length of the building, dividing the barn into 2 halves, and there were 2 wire-mesh partition walls installed across the width of the barn to further divide the barn into 6 distinct pens to control even bird distribution. The barn construction was typical for an Ontario layer operation; insulated stud walls with sheet-metal siding and roofing and a poured cement floor (Table 1). A belt egg collection system connected the test barn with other barns. The barn was mechanically ventilated with air inlets located along the ceiling and ventilation exhaust fans on the exterior walls. The outside air temperature was 12°C on the trial day.

For both experiments, animal carcasses were composted on-farm posttrial, the methodology for which has been described (Dam et al., 2009).

Surgery

All surgical and experimental procedures were approved by the University of Guelph Animal Care Committee, and all studies were monitored by either or both of CFIA and OMAFRA personnel, as well as by animal welfare specialists and veterinarians working for the University of Guelph, CFIA, and OMAFRA.

Birds were surgically instrumented at least 24 h before gassing with sterile transmitters containing 2 biopotential leads for recording electroencephalographs (**EEG**) and electrocardiographs (**ECG**) as well as activity and body temperature (TL11M2-F40-EET, Data Sciences International, St. Paul, MN). Briefly, birds were premedicated with 1.5 mg/kg of IM butorphanol (Torbugesic, Fort Dodge, IA) and 0.5 mg/kg of IM meloxicam (Metacam, Boehringer-Ingelheim, Burlington, ON). Anesthesia was induced by face mask with isoflurane (Forane, Abbott Laboratories, St-Laurent, QC) in oxygen; birds were intubated with an uncuffed endotracheal tube (2.5–3.5-mm ID), placed in dorsolateral recumbency, and maintained on a mixture of isoflurane in oxygen, given to effect. The surgical site was the less-feathered ventrolateral neck, and this area was plucked. The surgical method used was adapted from Savory and Kostal (1997). Following aseptic preparation of the skin and draping for surgery, a short incision was made through the skin on the side of the neck of each bird, and a small pocket was created by subcutaneous tunneling for the transmitter. One pair of biopotential leads was tunneled subcutaneously up the neck to emerge at the back of the head, and the skull was prepared for drilling. Two holes were made in the skull, and the leads were anchored into position on the cerebral dura with sterile cyanoacrylate adhesive (Vetbond, 3M, St. Paul, MN). The interlead distance was approximately 1 cm. An ECG lead II configuration was established by anchoring the second set of biopotential leads in diagonal opposition across the thorax. Following verification of signals via receiver plates (RPC-1, Data Sciences International) birds were recovered in individual plastic cat carriers and maintained until trial start. Prestudy pilot studies demonstrated normal behavior and EEG patterns in birds within 24 h of surgery (data not shown).

Delivery of Liquid CO_2

Pressurized liquid CO_2 was supplied by bulk tanker trucks (Praxair Canada, Mississauga, ON) through a mixing unit with separate inlets for vapor and liquid CO_2 from the truck. A minimum of 2 outlet hose connections were located on the mixing unit to supply CO_2 to the manifolds in the barn. Liquid CO_2 administration into the system was preceded by approximately one minute of vapor administration to pressurize the system, and the system was purged of liquid CO_2 after completion of liquid administration by 3 min of vapor administration. This sequence is performed to avoid freezing of the distribution system. A specially engineered gas discharge manifold system was designed and constructed from copper to vaporize the liquid CO_2 and dissipate the gas stream into the barn (Figure 1). The manifold was constructed from a series 1.91-cm diameter copper pipes interconnected with copper T-joints

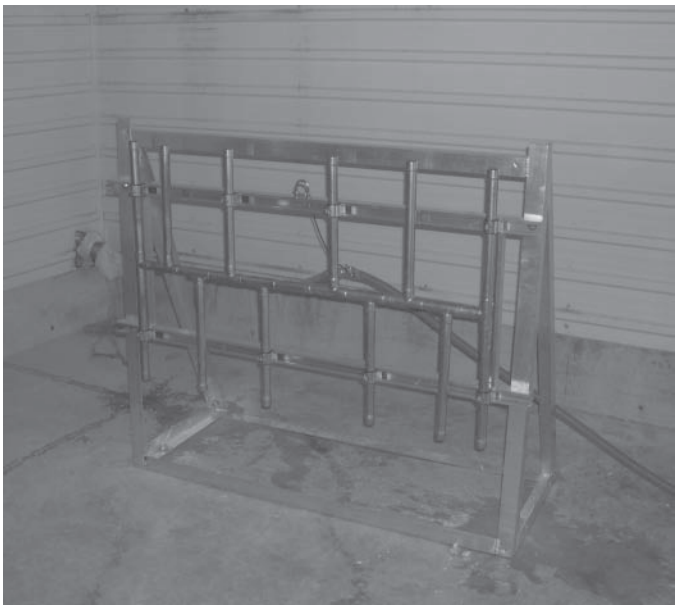

Figure 1. Copper manifold used for liquid CO_2 delivery in whole barn gassing. Manifold was stabilized with concrete blocks before use.

from a central trunk to form branches. Each branch was capped with a copper end cap. All joints were brazed to withstand gas pressure and prevent leakage. A total of 60 evenly spaced 1.59-mm diameter orifices were drilled into the copper pipes to vaporize the CO_2. The manifolds were connected to the mixing unit located outside the barn with flexible 1.91-cm diameter hoses that were rated for 15.51 MPa (2,250 psi) and cold temperature. Special safety cables were attached to cable ends to protect workers in case a pressurized hose disconnected from the mixing unit or discharge manifold.

For all barns, large openings to the hen housing area, including the manure and egg belt exits, were filled with fiberglass insulation batts and sealed using plastic sheeting (6 mil plastic vapor barrier) and duct tape. All windows, doors, and fans were similarly sealed with plastic sheeting and taped. Attic and upper sidewall air inlet openings were not sealed to allow ambient barn air to escape and to avoid any potential damage if the barn became pressurized. Because carbon dioxide is heavier than air and has a colder ambient temperature, it tends to fall to the lowest point, displacing warmer air upwards. Attic seals are not required to maintain CO_2 concentrations at the level of the hens.

For the empty barn pilot study, 2 manifolds were used and were located in 2 doorways in the side of the barn. For the 2 depopulation trials, 2 manifolds were placed at the front of the barn and 2 additional manifolds were placed approximately 150 ft (45.7 m) down the outside aisles of the barn.

In all cases, barn exhaust fans were left running until study start. These fans were covered with plastic from the outside, which could be removed safely after completion of CO_2 administration to allow for venting before personnel entry. Depending on the barn configuration, the time for venting varied, but no personnel were allowed to re-enter until oxygen readings inside the barns exceeded 20%. In both trials with hens, barn lights were turned off before initiating CO_2 delivery.

Gas and Temperature Monitoring

During the trials, environmental gas (CO_2 and O_2) concentrations and temperatures within the barns were monitored remotely using an Eagle 6-gas analyzer (RKI Instruments, Union City, CA) that was capable of measuring CO_2 levels from 0 to 60% by volume and a Draeger X-AM 7000 multigas monitor (Draeger Safety Canada Ltd., Mississauga, ON) configured to measure CO_2 levels up to 100% by volume. Gas levels were sampled in at least 2 places within each barn using flexible plastic tubing connected to the analyzers. Temperature was monitored using an Omega HH501AT unit (Omega, Laval, QC) with type T thermocouple. During the pilot study, gas concentrations were measured at 3 points in the barn to determine if the barn would act as one airspace or if differences in concentrations would occur. For experiment 1, barn temperature and gas concentrations were recorded in the first third of the barn, equidistant between the front manifolds and back of the barn, and close to where the instrumented birds had been placed. For experiment 2, the temperature and gas levels were recorded approximately 1.83 m above the barn floor, over the birds being monitored, which were placed in a middle enclosure and close to the ceiling of the barn at the end furthest from the manifolds.

For safety reasons, personnel standing near the barn during the trial and entering the barn after exhausting were equipped with MultiRae personal gas monitors (Rae Systems, San Jose, CA).

Behavioral Monitoring

For experiment 1, behavior of instrumented birds (chickens 1–4) was continuously recorded throughout the gassing trials by infrared videography (ThermaCAM EX320, FLIR Systems Canada Ltd., Burlington, ON; Figure 2). Behavioral activity monitored included agitation, head shaking, gasping, change in posture, and clonic muscle spasms. Time to loss of posture is directly correlated with time to unconsciousness (Gerritzen et al., 2006a). Activities were time-logged and correlated with the results of the physiologic data collected by telemetry as well as O_2 and CO_2 gas concentrations. Observers standing outside the barn during experiment 1 also recorded hen vocalizations.

Biotelemetric Data Acquisition and Analysis

Gross locomotor activity, body temperature, heart rate and rhythm (ECG), and cortical EEG were monitored telemetrically using Dataquest A.R.T. 4.0 software (DSI, St. Paul, MN). Implanted transmitters emitted frequency modulated signals received by an antenna board attached to each bird's cage. Locomo-

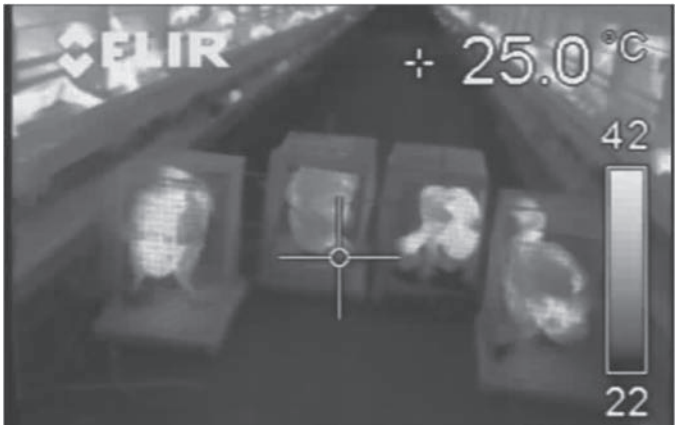

Figure 2. Infrared image of instrumented hens in cages on telemetry plates placed in aisle between 2 rows of caged layers before CO_2 gassing in experiment 1. Gradient bar on right indicates object temperature.

tor activity, body temperature, and ECG data were sampled in 10-s epochs and collapsed into 30-s bins for analysis. Raw data for each bird was also evaluated for signal interference or artifact, qualitative abnormalities in heart rhythm and pattern, and normal, abnormal, and isoelectric EEG activity (flat EEG tracing). The EEG raw data was subjected to fast Fourier transformation using continuous but nonoverlapping 1-s epochs (Otto, 2008) and a 2–32 Hz bandpass.

Baseline information was collected from resting birds within the barns on the day of the trials and parameters were recorded continuously throughout. Locomotor activity was individually evaluated in parallel with behavioral data collected by infrared videography, as described. Body temperature of instrumented birds was recorded continuously before and during each trial.

Video recordings, locomotor activity, and EEG amplitude were correlated to approximate time to unconsciousness in birds, and EEG recordings were used to determine time to brain death. A power spectrum analysis was applied and quantitative EEG variables calculated included median frequency (F50), spectral edge frequency (F95), and total power (Ptot; reviewed by Otto, 2008). Increases in EEG amplitude (Ptot) are associated with anesthetic induction (Murrell and Johnson, 2006; Gibson et al., 2007; Murrell et al., 2008). Unconsciousness in chickens has been associated with a change in baseline EEG to profound low frequency waves (decreases in Ptot, F50, and F95; Raj et al., 1998).

Values are presented as mean ± SE of results from individual birds (n = 4 or 8) in each trial.

RESULTS

Gas Concentrations and Ambient Barn Temperatures

Pilot Study. Over the period of administration, the CO_2 concentrations inside the barn rose from 0 to 60%, the O_2 concentration fell from 20.9% to 7.3%, and the air temperature dropped from 25.6°C to a minimum of −59°C. Temperatures were initially lower at monitoring points in line with the CO_2 gas stream. The rise in CO_2 concentrations was linear over the duration of gas administration. After the gas flow was stopped, CO_2 concentration decreased slightly over the next 90 min and the concentrations of oxygen continued to fall to a low of 4.7% after 40 min, remaining stable until venting. Despite the low temperatures, no dry ice (solid CO_2) accumulation was present when the space was re-entered.

Experiment 1. Temperature recordings were initiated 100 s after the liquid CO_2 was turned on, and at that time, the ambient temperature was 11.1°C (Figure 3a). The lowest barn temperature recorded was −23°C, occurring approximately 13.5 min after initiating liquid CO_2 delivery, just before turning off the flow. The CO_2 gas concentrations rapidly rose from 0.6%, exceeding 20% at 14 s after gas on (Figure 3b; Table 2). The CO_2 levels exceeded 60% at maximum and remained stable for the duration of recording, before gas venting. The exact level could not be determined because it exceeded the upper limit of the monitor being used. Oxygen concentrations started at 23% and never fell below 7.4% for the duration of the trial.

Experiment 2. The ambient temperature in the barn before study initiation was 26.3°C (Figure 4a). The temperature fell gradually after gas on and the lowest temperature recorded was 15.0°C, occurring 19 min after stopping the CO_2 flow. The CO_2 concentrations reached 20% at 12 min after gas on, with a maximum CO_2 level of 47% (Figure 4b; Table 2). Oxygen concentrations measured at the back end of the barn furthest from the manifolds started at 23% and fell to 13.1% at 32 min after gas on.

Body Temperatures

The core body temperatures of monitored birds in both experiments remained at or near normal while birds were alive. In experiment 1, the mean (SE) body temperatures dropped to 29.3 (7)°C by 20 min after gas on (Figure 5a; Table 2), whereas in experiment 2, the mean (SE) body temperature at 20 min after gas on was 38.7 (0.1)°C (Figure 5; Table 2). A walk-through of the barns after venting indicated that some eggs on the egg conveyor near the manifolds at the front of the barns had frozen during the gassing. There was no evidence of ante mortem hen freezing.

Bird Behaviors

In experiment 1, chickens 1 to 4 were videotaped using infrared thermography whereas regular videography was used for chickens 1 to 4 in experiment 2. Head shaking and open-mouthed breathing or gasping were noted in instrumented birds within the first 40 s as CO_2 levels around the birds began to rise. One bird in experiment 1 was also noted to defecate during this early

Table 2. Summary of gas and behavioral time data for whole barn depopulation of laying hens by liquid CO_2

Time measurement	Experiment 1[1]	Experiment 2[1]
Mean (SE) time to behavioral changes after gas on (s)	42 (0.4)[2]	n/a[3]
Mean (SE) time to induction after gas on (s)	80 (4.1)	n/a
Mean (SE) estimated time of death of instrumented birds (min:s)	5:01 (0:14)	5:06[4]
Mean (SE) body temperature of instrumented birds at estimated loss of consciousness (°C)	38.3 (0.6)	39.6 (0.2)
Time to 20% CO_2 after gas on (min:s)	00:40	8:06
Maximum recorded CO_2 concentration (%)	>60	47
Time to maximum recorded CO_2 concentration (min:s)	7:14	32:06
Time to end of barn vocalization or cage noise (min:s)	n/a	10:30

[1]n = 8 monitored birds for experiment 1, n = 4 for experiment 2.

[2]Based on video recordings and activity patterns collected by telemetry.

[3]n/a = not available.

[4]Based on heart rate and electroencephalograph waveform changes.

exposure period. Wing stretching or flapping attempts and up to 2 myoclonic contractions were also seen in some videotaped birds in both trials before the onset of unconsciousness, which occurred less than 2 min after initiating CO_2 flow. Whole body relaxation was readily observed at the end of induction and onset of unconsciousness, with additional sporadic but vigorous myoclonic contractions. One hundred percent mortality was achieved in both trials and no piling of free-range birds was noted in any of the 6 enclosures in experiment 2, with even dispersion of dead birds over the floor and perching areas.

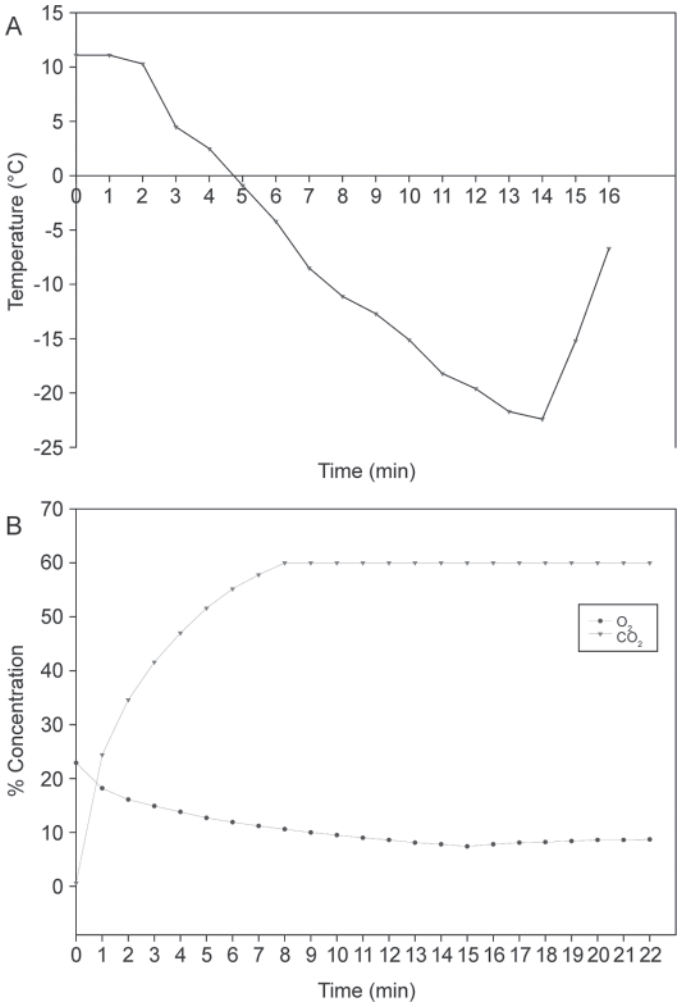

Figure 3. A) Ambient barn temperature changes with time after liquid CO_2 was initiated for experiment 1 (caged layers). B) Changes in CO_2 and O_2 concentrations with time in barn after liquid CO_2 was initiated at time 0 for experiment 1.

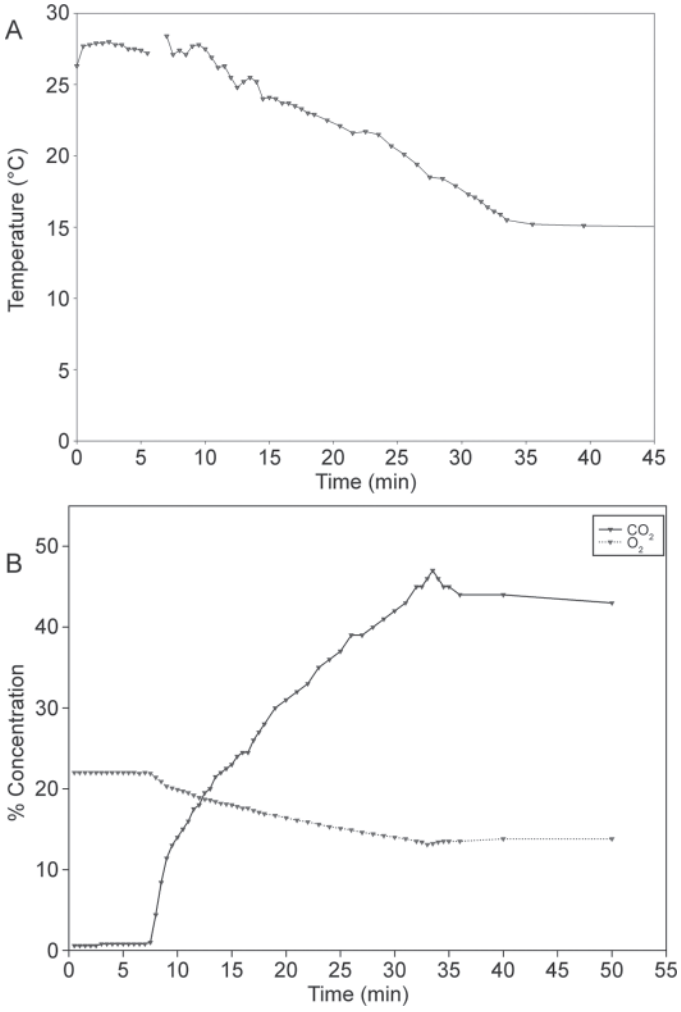

Figure 4. A) Ambient barn temperature changes with time after liquid CO_2 was initiated for experiment 2 (free-range layers). B) Changes in CO_2 and O_2 concentrations with time in barn after liquid CO_2 was initiated at time 0 for experiment 2.

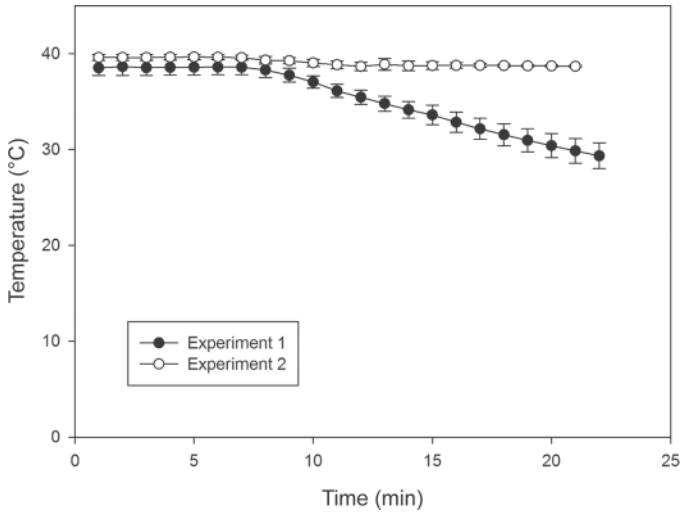

Figure 5. Mean (SE) body temperature of hens with time during CO_2 gassing for experiment 1 (caged layers, n = 8) and experiment 2 (free-range layers, n = 4).

Heart Rate and ECG Findings

In both experiments, there was a mild but nonsignificant increase in heart rate from baseline within the first few minutes after gas onset (Figure 6). Abnormalities in sinus rhythm were seen consistently within the first 2 min after CO_2 was turned on. In experiment 1, first degree heart block was noted in 5 of 8 birds within 6 min (56% CO_2) and ECG changes consistent with acute myocardial infarction and hypoxia (premature ventricular contractions, atrial fibrillation, and elevated S-T segment) were noted in all animals by 7 min (58% CO_2) after the liquid CO_2 was turned on (Brandt et al., 1998; Sgarbossa et al., 2001; Figure 6a). In experiment 2, the mean heart rate dropped precipitously in all 4 chickens between 6 to 6.5 min after the liquid CO_2 was turned on, changing from 142 ± 16 to 39.5 ± 0.3 beats per minute (Figure 6b), and it never recovered. A flat-line ECG was noted in all 8 chickens in experiment 1 by 11 min after gas on, correlating with 6.8% O_2 and at least 60% CO_2 levels.

EEG Findings

Time to unconsciousness was estimated from thermal videography in experiment 1, in which whole body relaxation (loss of posture) could be directly seen at the end of the induction period. Brain death occurred within approximately 5 min after turning the liquid CO_2 on in both studies. This was recognized by a marked decrease in frequency of waves (Figure 7a) when calculating total power, as well as reductions in the median and spectral edge frequencies (Figure 7b). A flat-line EEG consistent with brain death was also detectable from the raw EEG tracings and was used to confirm interpretation of spectral power analyses. Both methods suggested brain death at approximately 5 min after gas on in both experiments.

DISCUSSION

The pilot study results clearly indicated that it was possible to achieve appropriate concentrations of CO_2 to depopulate a poultry flock over a short period of time in a properly sealed barn, and that for practical purposes, a barn can be considered to be a single airspace even though barriers, such as nesting boxes, may be present. The subsequent bird studies demonstrate that end-of-lay hens held in either free-range or caged conditions can be rapidly and safely killed with 100% mortality using whole barn liquid CO_2 and a special manifold system for delivery of the vaporized liquid. All vocalizations in the larger barn (experiment 2) stopped less than 11 min after the liquid CO_2 delivery was initiated and instrumented birds being monitored by videography (experiment 1) became unconscious, as measured by changes in EEG activity and by marked postural changes, at less than 2 min, correlating with ambient CO_2 levels of 18% to 20% in that part of the barn. Cardiac abnormalities developed as CO_2 levels and hypoxia

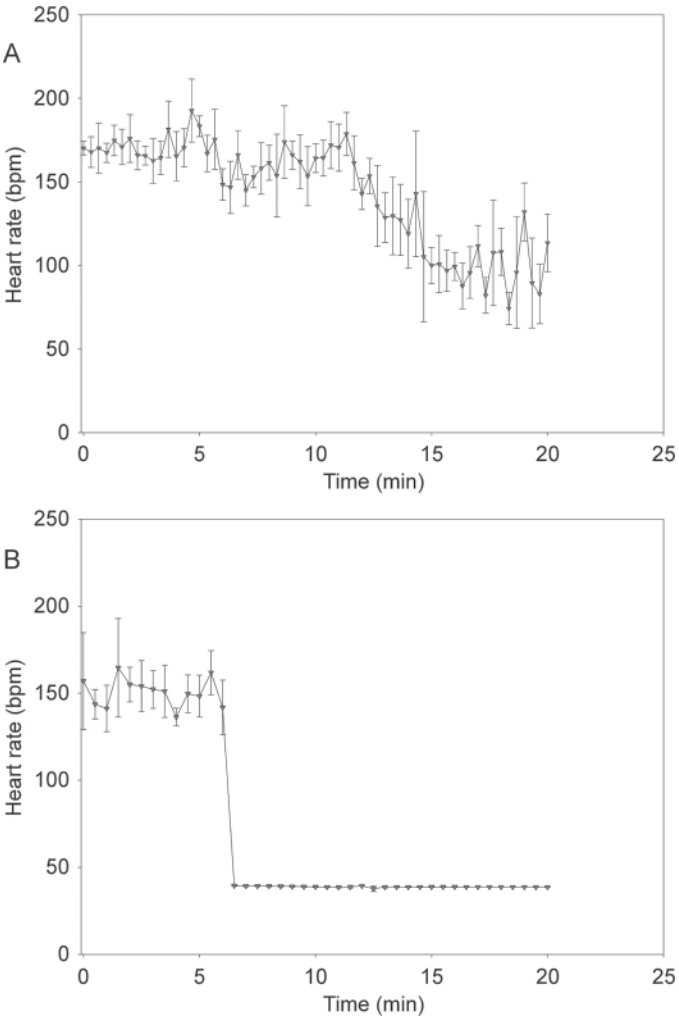

Figure 6. A) Mean (SE) heart rate of hens with time during CO_2 gassing (n = 8) for experiment 1 (caged layers). B) Mean (SE) heart rate of hens with time during CO_2 gassing (n = 4) for experiment 2 (free-range layers).

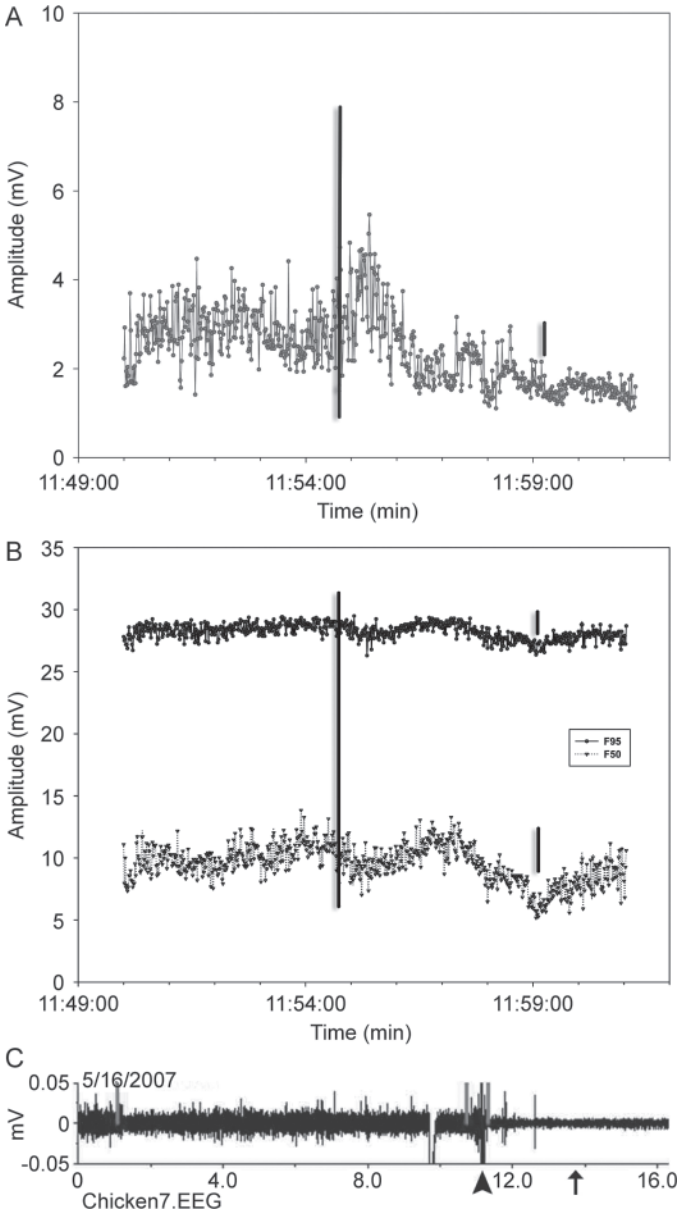

Figure 7. A) Mean (SD) electroencephalograph (EEG) total power during CO_2 gassing (n = 8) for experiment 1 (caged layers). B) Mean (SD) EEG F95 (spectral edge frequency) and F50 (median frequency) of hens during CO_2 gassing (n = 8) for experiment 1 (caged layers). Long bar represents gas on, short bars indicate significant amplitude changes in F50, F95, and Ptot (increases in EEG amplitutde) within 5 min of gas on, indicative of brain death. C) Example of raw EEG tracing from hen in experiment 1. First gray vertical bar represents time of the baseline EEG reading after hens were in place. Second gray bar indicates time that CO_2 was turned on. Third gray bar represents time to 20% ambient CO_2 concentration. Note the rapid decrease in EEG amplitude shortly after exposure to CO_2 gas. Arrowhead indicates whole body contraction and arrow represents time to estimated EEG flatline.

increased and brain death of the instrumented animals at the front of each barn occurred approximately 5 min after turning the gas on. Heart beating was observed well after brain death; however, analysis of the waveforms demonstrated arrhythmias inconsistent with coordinated pumping of blood. There was no postgassing evidence of birds being frozen before death, and ambient barn temperatures were maintained between 11 and 27°C during the period of time that birds were alive and conscious. A change in ambient temperature gradient by the advancing CO_2 gas was readily visualized by infrared thermography as a black wave moving close to the ground. The caged hens above the level of the gas wave appeared to watch this move by but it is unknown whether chickens can visualize infrared spectra. Their concurrent behavior was quiet and not indicative of distress. Vocalizations only occurred as the CO_2 gas rose around and enveloped the birds.

Use of liquid CO_2 led to mildly reduced ambient temperatures in the barn during the period of hen consciousness, but no feasible method was found to heat the CO_2 to ambient temperature. The technologies examined were not of sufficient scale or dependability to be useful, and it was felt they were unacceptable due to risks of failure and prolongation of the administration phase. It is important to ensure that the manifolds are placed such that vaporized gas is not sprayed directly onto animals to minimize exposure to uncomfortably low temperatures.

The findings from this study are consistent with results seen when either end-of-lay hens or broiler chickens are stunned or euthanatized by CO_2 inhalation in small chamber controlled atmosphere environments (Webster and Fletcher, 2001; Gerritzen et al., 2004; Kingston et al., 2005; McKeegan et al., 2007). Typically, higher CO_2 levels (initially 35–45%) are used for stunning-kill procedures in abattoirs because of the shorter exposure period of birds to gas before exsanguination. Gustatory responses (mandibulation) and head shaking are reported at 10% CO_2 levels, which are lower than those required for trigeminal nerve nociception (McKeegan et al., 2005), suggesting that oral acidic stimulation may be the first behavioral response noted following CO_2 exposure in chickens. The interpretation of head shaking behavior in response to CO_2 in chickens is unclear, and some have suggested that it indicates aversion (Webster and Fletcher, 2001), whereas others maintain that it is an alerting response (Hughes, 1983). It is not a dose-dependent effect, however, and exposure of broiler chickens to 10 s of 25% CO_2 induces minimal food withdrawal, which is more consistent with the concept that low levels are stimulatory but not necessarily strongly aversive to chickens (McKeegan et al., 2006). Association of only mild, nonsignificant increases in heart rate in this study with increasing levels of ambient CO_2 suggests that hens were not significantly distressed during the induction phase. Violent wing flapping has been reported with sudden exposure to high levels of CO_2 or CO_2-inert gas mixtures, and this is attributed to tonic-clonic seizure activity (McKeegan et al., 2005). It is unclear in our study whether the wing stretching noted in the instrumented birds was related to CO_2-induced muscle acidosis or was a response to counter loss of balance during hypercapnea induction. Finally, no piling of birds was noted in the free-range barn experiment,

consistent with rapid induction of unconsciousness and minimal escape attempts, further suggesting that significant CO_2 aversion did not occur.

It is impossible to accurately define time to loss of sensibility in hens during gassing; however, correlation of major postural changes (whole body relaxation or loss of posture) with decreased brain electrical activity have been used as surrogate markers of unconsciousness (Gerritzen et al., 2004; Raj et al., 2006). Both loss of posture and a reduction in α:delta brain wave ratios have been directly correlated with loss of unconsciousness in chickens and either may be used interchangeably to determine when this state occurs (Benson et al., 2012). Further, increases in Ptot and decreases in F50 and F95 have been associated with decreased nociception and decreased cortical activity in various species (Murrell and Johnson, 2006; Gibson et al., 2007) and were noted in the current study. In our study, estimated loss of consciousness in end-of-lay hens consistently occurred at ambient CO_2 levels of 18 to 20% and O_2 levels <20%. This is similar to observations by Gerritzen et al. (2004) when evaluating CO_2 gas in chambers with broiler chickens, in which loss of posture occurred at 13% CO_2 and isoelectric EEG were obtained at 20% CO_2. In the present study, changes in power spectral analyses were correlated with postural changes noted by direct thermal videography in experiment 1. A shortfall of experiment 2 was that gas sensor probes were located above the heads of the instrumented hens and thus recordings of ambient gas levels are likely lower than the actual CO_2 levels experienced by the birds at the ground level. A slower rise in ambient CO_2 levels during whole barn gassing may have resulted in lower stress levels experienced by hens in this study, similar to observations noted for other species, such as rodents (Neil and Weary, 2006). Exposure to increasing levels of CO_2 exceeding 20% for at least 5 min was required to ensure irreversible brain death in these hens. Prolonged electrical activity of the heart after brain death is reported in chickens and other species (Conci et al., 2001; Dawson et al., 2007), thus presence or absence of a heartbeat may not accurately predict time of death. Sporadic, uncoordinated, and irregular ECG patterns were consistent with increasing hypoxia and myocardial ischemic infarction and lasted up to 11 min in some instrumented hens.

Calculation estimates for the volume of CO_2 gas required for whole barn depopulations have been described and are determined by multiplying barn volume by both the target level of CO_2 desired and a conversion factor that describes the change in volume of liquid CO_2 to its gaseous state; that is, 0.45 kg or 1 lb of liquid CO_2 generates 0.25 m^3 (8.75 ft^3) gas at 1 atm pressure and 21°C (Kingston et al., 2005). This estimate results in roughly a 1:1 correlation between barn volume and volume of CO_2 gas required for depopulation, assuming a target CO_2 level of 45%. In addition to the 2 barns described in detail in this study, this estimate has been used successfully by the CFIA for whole barn depopulations at 7 other sites across Canada (H. Kloeze, CFIA, Ottowa, Canada, unpublished data). Although target CO_2 concentrations of 45 to 60% are the goal, it can be difficult to achieve an airtight barn seal before gassing and a full seal is not necessary to achieve reliable results. Experience from these and other trials indicates that it is important to effectively seal openings at the bottom of the barn, such as water drains, manure belt openings, and doorways, and all major openings in the walls of the barns to minimize CO_2 leakage. It is clear from the current study that lower CO_2 levels may also be rapidly efficacious in killing large numbers of birds, particularly when birds are housed under free-range conditions, because CO_2 is heavier than air. However, erring on the side of excessive CO_2 levels is always advisable to ensure rapid and humane death of all animals.

Welfare concerns for whole barn gassing with CO_2 or other inert gasses have been recently summarized (Raj et al., 2006). As seen in this study, some welfare concerns were noted and included early signs of respiratory stimulation and vocalization during CO_2 induction; death was not instantaneous, and in the larger barn, it took up to 10.5 min for all birds to be rendered unconscious after initiating pumping of liquid CO_2. And 1 to 2 clonic contractions occurred in hens before the onset of unconsciousness. These concerns must be balanced carefully with human safety and animal welfare concerns seen with traditional methods of hen depopulation. For situations in which depopulation must occur because of infectious disease concerns, for example, the presence of reportable diseases, such as exotic Newcastle disease or highly pathogenic avian influenza, whole barn gassing clearly optimizes biosafety by minimizing contact between handlers and live birds. The barn can be sealed and manifolds placed with minimal entry of personnel into the barn space and sprayed with disinfectant postgassing, and carcasses and litter left in situ until decontamination has occurred. For routine depopulation of end-of-lay hens, we believe that whole barn gassing may offer several significant improvements for animal welfare, particularly for situations in which animals must be transported long distances for slaughter and processing. There is no need for food or water withdrawal before gassing, animals are maintained in a familiar environment; and no handling is required, an important consideration because animal catching and handling may be stressful to birds (Yalcin et al., 2004) and because of the fragile skeletons of hens at end-of-lay, resulting in inadvertent fractures of up to 29% of caged hens during loading and unloading (Gregory and Wilkins, 1989; Knowles and Wilkins, 1998). Lengthy transportation times (potentially exceeding 24 h) and wait times at slaughter facilities may also be significant sources of stress for cull hens and are eliminated with whole barn gassing. On balance, when weighing the costs and benefits for animal welfare, large welfare improvements may be realized with whole barn CO_2 gassing of laying hens.

Table 3. Whole barn CO_2 gassing—direct producer estimated costs[1]

Item	Cost ($)
Liquid CO_2 (gas purchase, delivery, standby)	4,210.00
Barn preparation (sealing ventilation—labor and materials)[2]	240.00
Purchase or rental of manifolds/hoses (assumed 3)	715.00
Crew removing dead birds from barn[3]	1,250.00
Total	6,415.00
Cost/bird	$6,415.00/30,000 = $0.21/bird

[1]Based on Ontario charges and estimates for a 30,000 caged layer barn (June 2011).

[2]Will vary slightly based on barn size and ventilation configuration, assuming a crew of 4 people working for 2 h each at $15.00/h.

[3]Producer will also have additional costs associated with disposal of carcasses. Options include burial, composting, and pick-up by rendering company. Additional disposal costs of $0.15–0.30/bird are estimated depending on farm location and availability of materials (for example, carbon source for composting).

To be implemented widely in commercial settings, a major consideration is the direct financial cost to the producer, and there are several considerations impacting this. Because of the potential for adverse human health and animal welfare outcomes if not performed correctly, this is not a technique that producers should perform without assistance. At minimum, a specialist with training and experience with this technique should be present every time to supervise barn preparation; oversee placement of manifolds, CO_2 delivery, and measurement of in-barn gas concentrations; ensure adequacy of the procedure in terms of rapidly inducing 100% mortality of hens; and guarantee human safety by controlling access to the barn and ensuring appropriate venting and safe CO_2 levels in the barn before any human re-entry. All on-site personnel must be accounted for before sealing the barn and initiating liquid CO_2 pumping. In addition, a technician from the CO_2 supply company must be present to control the delivery. Preparation time for gassing will vary depending on the size of the barn but 4 trained persons may require up to 2 h for larger barns, potentially necessitating hiring additional personnel to assist with procedures. Some economy of scale may be realized, however, because costs will be distributed over more animals in larger operations. A direct cost analysis for whole barn gassing (Table 3) suggests that whole barn gassing is more expensive for producers. In Canada, producers pay for catching and loading of cull hens onto trucks whereas the processor pays for transportation of cull hens to slaughter facilities; transportation costs being directly dependent on the distance traveled. A relatively short distance (3 h) was used for this transportation estimate, thus costs will increase with longer transport distances. These overall costs must also be balanced with the improvements to animal welfare that may be realized. Thus use of whole barn gassing may be an economically viable means for routine depopulation of end-of-lay hens. Additional economies may be realized by on-farm composting of carcasses to reduce fertilizer costs.

In conclusion, this study has demonstrated that liquid CO_2 delivery to whole barns is a viable means of depopulating layer hens in the face of foreign animal disease outbreaks and may be adapted for routine depopulation of end-of-lay hens. Some welfare concerns are noted for this method, including signs of respiratory irritation and muscle acidosis (clonic muscle contractions) before loss of consciousness; however, loss of consciousness is rapid after CO_2 induction. It is essential that proper safety procedures be in place when using this technique and that the procedure be appropriately supervised to ensure rapid loss of consciousness of hens while minimizing risks to personnel.

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