Identifying Process Variables for a Low Atmospheric Pressure Stunning-Killing System

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Primary Audience: Poultry Processors, Researchers

SUMMARY

Current systems for preslaughter gas stunning and killing of broilers use process gases such as CO₂, N₂, Ar, or a mixture of these gases with air or O₂. These systems, known as controlled-atmosphere stunning-killing systems, work by displacing O₂, ultimately to induce hypoxia in the bird, leading to unconsciousness and death. In this study, mechanical removal of O₂ by rapidly reducing air pressure was investigated as an alternative to controlled-atmosphere stunning-killing systems. Low atmospheric pressure systems could offer advantages in worker safety and operational gas cost because they operate solely with atmospheric air. This study comprised 2 experiments, one to define the initial range of effective pressures, and the second to determine a recommended process pressure. In experiment 1, 48 female broilers, aged 63 d, were subjected to 6 different pressure treatments, ranging from 70.9 to 17.8 kPa. In experiment 2, 56 male broilers, aged 60 d, were subjected to 7 different pressure treatments, ranging from 35.3 to 17.8 kPa. Birds were individually placed in an airtight vessel and exposed to a pressure treatment for 2 min after the final pressure was attained. Results from experiment 1 showed that the effective range of pressure was between 29.5 and 17.8 kPa, with only 25% of the birds exposed to 29.5 kPa surviving and none of the birds exposed to 17.8 kPa surviving. Experiment 2 used a finer resolution of pressure increments, and the estimated pressure level lethal for 99.99% of the birds was determined to be 19.4 kPa.

Key words: broiler, gas stunning, slaughter

DESCRIPTION OF PROBLEM

Previous research focusing on the development and optimization of gas stunning-killing systems for broilers has used process gases such as CO₂, N₂, Ar, or a mixture of these gases with air or O₂ to incapacitate hens and broilers prior to shackling and exsanguination [1, 2, 3]. However, birds can rapidly recover from exposure to these gas mixtures; therefore, a stun-to-kill process has been recommended [2, 4, 5]. Unconsciousness was reported in hens when the O₂ concentration was reduced below 5% O₂ by volume [6], and less than 2% O₂ by volume is recommended for anoxic stun-to-kill processes [7, 8]. However, in gas mixtures containing CO₂, unconsciousness may be induced when higher concentrations of O₂ are present with an extended exposure time [9, 10, 11].

One limitation of gas stunning systems is in achieving uniform concentrations of gases in the

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atmosphere surrounding the birds as a consequence of inadequate mixing, which may lead to pockets of air between the birds, reducing the effectiveness of the process and prolonging the process time [7]. Mechanically removing air to reduce the atmospheric pressure, thereby reducing the partial pressure of oxygen (\(P_{O_2}\)), may be an alternative to controlled-atmosphere stunning-killing systems, because pressure is exerted uniformly in a vessel and does not depend on the type of process gas used. The use of reduced atmospheric pressure as a means of slaughter has been approved for use in farmed game species (quail, partridge, and pheasant) in Europe [12]. However, little information exists on the responses of broilers to reduced atmospheric pressure. Thus, the objectives of this study were to 1) determine the operating range of atmospheric pressures, and 2) to determine the optimum pressure level required to reliably stun and subsequently kill broilers by inducing irreversible cessation of respiratory ventilation movements.

**MATERIALS AND METHODS**

**Test System**

The test system was composed of an 83.3-L cylindrical vessel [13] connected directly to a rotary vane vacuum pump [14] with a flow rate of 16.9 m\(^3\)/h; the vessel was equipped with a translucent acrylic lid for observation. A PC-based data acquisition and control system [15] was used to monitor tank pressure and control pump operation, and tank pressure was measured with a strain gauge-based pressure transducer [16]. Inlet and exhaust airflow was routed by 2 manually actuated ball valves. During the tests, the inlet valve was closed to isolate the tank from the external atmosphere, and airflow was directed through the pump via the second valve. At the conclusion of the test, the tank was returned to atmospheric pressure through the inlet valve. The experiments in this study were approved by the animal care and use committee at the USDA-ARS Mississippi State location.

**Experimental Design**

The upper pressure level (70.9 kPa) was selected based on the allowable range for human habitats in long-term space exploration [17], and the lowest level (17.8 kPa) was selected based on \(O_2\) partial pressures shown to induce unconsciousness in hens [6]. Birds in both experiments were exposed to the respective treatments for 2 min after final pressure had been attained, and in each of the 2 experiments, 8 replications per treatment were used.

In experiment 1, 48 Ross \(\times\) Ross 708 [18] female broilers, aged 63 d, were individually subjected to the following target pressure levels: 70.9, 60.8, 50.7, 40.5, 29.5, and 17.8 kPa. Pressure levels used in experiment 2 were taken from the lower range of pressures in experiment 1 that resulted in loss of posture (LOP). With these pressures from experiment 1, 56 Ross \(\times\) Ross 708 male broilers, aged 60 d, were individually subjected to target pressure levels of 35.3, 32.1, 29.5, 26.6, 23.6, 20.7, and 17.8 kPa.

Loss of posture, resulting from the inability to maintain a sitting position or neck tension, has been noted to occur at the onset of unconsciousness [19, 20]. The occurrence of LOP and cessation of respiratory ventilation movements were recorded in experiment 1 as primary responses of interest from which to determine the range of operating pressures; a bird was considered dead when respiratory ventilation movements had ceased. Movement of the keel bone was used as the major indicator of respiratory ventilation movement. Elapsed times to LOP and cessation of respiratory ventilation movements were also recorded in experiment 2 and were determined with a stopwatch.

**Statistical Analysis**

Loss of posture and survival were coded as binary data (occurrence = 1; no occurrence = 0). Binary data were compared by using logistic regression [21, 22] with PROC GENMOD [20]. Time data were compared by using ANOVA with PROC MIXED [23]. Dose-response relationships can be described by a sigmoid-shaped curve [24] of the form (equation [1]):

\[
y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b}
\]

where \(y\) is the probability of survival, \(a\) is the asymptotic maximum probability, \(x\) is the slope of transition (1/kPa), \(x_0\) is the midpoint of transi-
Table 1. Effect of atmospheric pressure set point on incidence of loss of posture (LOP) and mortality (experiment 1)

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>Incidence of LOP (%)</th>
<th>Incidence of mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>29.5</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>40.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>70.9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

tion (kPa), and $b$ is the inflection point for the asymptotic maximum/minimum.

Survival data were fitted to this equation, and nonlinear regression analysis was used to determine the dose-response relationship between atmospheric pressure and bird responses [25]. Statistical significance was established at $P \leq 0.05$.

**RESULTS AND DISCUSSION**

**Experiment 1**

Data from experiment 1 are shown in Table 1. The higher pressure treatments (70.9, 60.8, 50.7, and 40.5 kPa) elicited no responses of interest from broilers in this experiment and were subsequently excluded from further experiments. Loss of posture was observed in all birds exposed to the remaining 2 treatments (29.5 and 17.8 kPa). Of those birds, 75% exposed to 29.5 kPa and 100% exposed to 17.8 kPa did not survive the treatment. These data showed complete separation, and no further statistical analysis was warranted. The pressure treatments used in experiment 2 were subsequently selected to include this range.

**Experiment 2**

The goal of experiment 2 was to determine the optimal operating pressure for a low-pressure system. As observed in experiment 1, the proportion of birds surviving and exhibiting LOP increased with increasing atmospheric pressure. Time to LOP ranged from 34.1 to 50.5 s, and means are presented in Table 2. The times to LOP for the lowest 4 pressures ($\leq 26.6$ kPa) are very similar, ranging from 34.1 to 34.9 s. Time to death increased with increasing atmospheric pressure and ranged from 79.1 to 142.8 s (Table 2). Time to cessation of respiratory ventilation movement was not different between pressures $\leq 23.6$ kPa; however, these 3 set points differed from the 26.6- and 29.5-kPa set points ($P \leq 0.01$).

Survival data from experiment 2 were fitted to equation [1], and the resulting coefficients were recorded: $a = 93.3672$ ($P < 0.0001$), $b = -21.1147$ ($P = 0.01$), and $x_0 = 29.8918$ kPa ($P < 0.0001$), with an SE of 4.2. By using this equation, we could determine the pressure resulting in the desired proportion of mortality; for 99.99% mortality, a maximum pressure of 19.4 kPa should be used.

Time to LOP in experiment 2 for birds exposed to pressures $\leq 26.6$ kPa fell within a narrow range, showing little correlation with atmospheric pressure. This resulted from the nature of the pump-down cycle of the test system. With a constant flow rate and vessel volume, evacuation of the system was highly repeatable, and the vessel reached the same pressure, with little variation in time. Pressure measurements were monitored and recorded throughout the pumping and holding phases (Figure 1). Taking the average time to LOP for the lowest 4 pressures (34.5 s) and then determining the pressure at that stage during the pumping phase, an estimate of the pressure at which LOP occurs could be determined. For all birds ($n = 32$) in these 4 treatments, the average pressure after 34.5 s of evacuation was 21.1 kPa, with an SE of 0.8.
Table 2. Effect of atmospheric pressure set point on incidence of loss of posture (LOP), time to LOP, incidence of mortality, and time to cessation of respiratory ventilation movement (experiment 2)\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>Incidence of LOP (%)</th>
<th>Time to LOP (s)</th>
<th>Incidence of mortality (%)</th>
<th>Time to death (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.8</td>
<td>100</td>
<td>34.5 ± 0.7\textsuperscript{c}</td>
<td>100.0</td>
<td>79.1 ± 1.6\textsuperscript{b}</td>
</tr>
<tr>
<td>20.7</td>
<td>100</td>
<td>37.9 ± 1.0\textsuperscript{c}</td>
<td>100.0</td>
<td>85.5 ± 1.5\textsuperscript{b}</td>
</tr>
<tr>
<td>23.6</td>
<td>100</td>
<td>34.1 ± 1.3\textsuperscript{c}</td>
<td>100.0</td>
<td>83.4 ± 3.8\textsuperscript{b}</td>
</tr>
<tr>
<td>26.6</td>
<td>100</td>
<td>34.6 ± 1.6\textsuperscript{c}</td>
<td>100.0</td>
<td>128.4 ± 8.3\textsuperscript{a}</td>
</tr>
<tr>
<td>29.5</td>
<td>100</td>
<td>38.1 ± 2.3\textsuperscript{bc}</td>
<td>62.5</td>
<td>142.8 ± 8.7\textsuperscript{a}</td>
</tr>
<tr>
<td>32.1</td>
<td>75</td>
<td>50.5 ± 5.4\textsuperscript{a}</td>
<td>12.5</td>
<td>3</td>
</tr>
<tr>
<td>35.3</td>
<td>75</td>
<td>46.7 ± 4.3\textsuperscript{ab}</td>
<td>12.5</td>
<td>3</td>
</tr>
</tbody>
</table>

\textsuperscript{a–c}Means within a column with no common superscript differ (\textit{P} < 0.05).

\textsuperscript{1}Birds were exposed to low-pressure conditions for 2 min after the final pressure was attained.

\textsuperscript{2}Table values represent mean ± SEM.

\textsuperscript{3}Time to death was excluded for these pressure settings because of the low incidence of mortality associated with them.

Raj and Gregory [7] recommended an O\textsubscript{2} concentration of 2\% by volume under nominal atmospheric pressure, equating to approximately 2.0 kPa P\textsubscript{O\textsubscript{2}}, to minimize problems that might arise from uneven gas distribution. Results of this study showed that for the case of reduced atmospheric pressure, reducing P\textsubscript{O\textsubscript{2}} to approximately 3.7 kPa at the lowest pressure of 17.8 kPa was sufficient to stun and kill broilers. The pressure required for 99.99\% mortality (19.4 kPa) would result in a P\textsubscript{O\textsubscript{2}} of 4.0 kPa, which is similar to that of other anoxic systems at nominal atmospheric pressure with good gas distribution. Mean time to death at pressures ≤23.6 kPa was 82.7 s and was similar to those reported for the Ar, CO\textsubscript{2}, and N\textsubscript{2} processes [9, 10, 26, 27].

The American Veterinary Medical Association lists decompression as an unacceptable means of euthanasia for animals, with the primary concern that pain and distress could occur from gases trapped within the body [28]. However, concerns regarding air trapped in the body cavity are not applicable to birds. The anatomy of the avian respiratory apparatus differs from mammals in many respects. Lungs are fixed and do not expand and contract; the lungs are attached to air sacs and ramifications of these air sacs extend into many bones. Air sacs completely fill all the vacant space in the thoracic cavity and much of the abdominal cavity as well. Birds have only a rudimentary diaphragm, which does not extend across the interface of the thoracic and abdominal cavities. Thus, in poultry, trapped air pockets in the body cavity are impossible because of the organization of the extensive respiratory apparatus. Fedde [29] provides an excellent discussion of the avian respiratory system.

A low-pressure system may provide economic and safety advantages over other gas stunning processes. The process uses atmospheric air, eliminating the need to purchase process gases or generate them on site. Safety concerns about worker exposure to process gases are eliminated, and any leaks would bring atmospheric air into the system, rather than discharging it. Further studies should address the influence of evacuation rate and exposure time to refine the process, determine the effects of this process on physiological responses and on carcass or meat quality, and evaluate the welfare aspects of using this process.

CONCLUSIONS AND APPLICATIONS

1. Controlled-atmospheric pressure reduction appears to be an effective method for humanely stunning and killing chickens.
2. The mean time to LOP was 34.5 s for pressures ≤26.6 kPa.
3. The mean time to death was 82.7 s for pressures ≤23.6 kPa and is similar to times reported for other controlled-atmosphere processes.
4. The estimated operating pressure for a low-pressure stunning-killing system was 19.4 kPa.

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