Research Note: Methods in detecting signs of life after gaseous stun in broilers

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ABSTRACT To be compliant with religious requirements for poultry, slaughter facilities using controlled atmosphere stunning may require a reliable method for detecting signs of life. However, the production setting challenges methods utilized in veterinary and field use by generating excess sound and vibration, which can impact the effectiveness of typical measurement tools, such as the stethoscope or doppler ultrasound. In addition, it is required that the process be quick and efficient as to not interfere with the rapid pace of production. The current study assessed various methods including reflexes (pupillary light reflex, nictitating membrane reflex, and pedal reflex), doppler ultrasound, stethoscopes, and pulse oximeter for determination of signs of life in broiler chickens postcontrolled atmosphere stunning in production. Data were analyzed using a generalized linear mixed model in SAS. Each bird was considered an experimental unit (n = 18) and differences between dead on arrival (**DOA**; n = 6), stunned (n = 10) and sensible (n = 2) birds were compared using contrast statements. Reflexes were seen only in sensible birds. Of the evaluated tools, only the pulse oximeter was consistently capable of detecting heart beats per minute (**bpm**) within the production environment. Although the values of bpm did not differ between sensible and stunned birds ($P = 0.724$), DOA birds had a lower bpm than all others ($P < 0.001$). Although the accuracy of results deserves further investigation and the small sample size of this study posed challenges to our statistical analyses, including low statistical power for the comparison between sensible and stunned groups, the achieved results suggest that the pulse oximeter meets the requirements for a reliable and practical method in detecting signs of life in broilers. With the increasing importance of halal products in a market that prioritizes animal welfare, our results present a promising approach to meet halal certification requirements. Further studies on this topic are strongly encouraged.

Key words: heart rate, poultry, halal, controlled atmosphere stunning, religious slaughter

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INTRODUCTION

Determining if an animal intended for human consumption is alive at the time of applied killing method is an important process for religious requirements of halal slaughter and must be ensured by any food processor supplying halal meat (Farouk et al., 2014). It has been regarded in survey that just under half of halal consumers consider meat as non-Halal when animals are stunned prior to being cut (Fuseini et al., 2017). However, with an increasing number of countries implementing full bans on traditional slaughter without stunning due to animal welfare concerns, a segment of Islamic scholars has indicated that stunning can be halal-compliant if demonstrated that it does not result in instantaneous death (Fuseini, 2019), which is provided by a reliable method to differentiate dead from live stunned birds. Due to its objective assessment and alignment with both scientific (Woods et al., 2010) and religious (Fuseini, 2019) descriptions of death, the presence of a heartbeat is often chosen as a measurable metric for determining life (Fuseini, 2019). However, the fast production pace and loud noise characteristics of industrial settings pose challenges to this assessment, requiring a reliable and practical method in detecting signs of life.

Controlled atmosphere stunning (**CAS**) systems, which have become increasingly popular across Canada and Europe, allow poultry to remain in transport modules until after gas stunning occurs. Although this system eliminates welfare concerns of excessive handling and conscious shackling (McKeegan et al., 2007), it introduces the need for a method of validating life in stunned birds when separating them from dead on arrival (**DOA**) birds during shackling. In the case of poultry, anatomical differences from mammals such as air sacs, the keel bone and elevated resting heart rate

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pose additional difficulties to the tools typically used for assessing signs of life.

In this study, we assessed various methods (reflexes, doppler ultrasound, stethoscopes, and pulse oximeter) for determination of signs of life in broiler chickens post-CAS exposure. Our choices were based on the practicability and reliability of each method. For example, although reflexes do not aid in identifying a heart rate, they measure the effectiveness of a processor's stunning method. Alternatively, classic stethoscopes can audibly detect heart rate, electronic stethoscopes and portable fetal doppler ultrasounds can detect heart rate and display beats per minute (**bpm**); whereas the pulse oximeter is a device which can detect and display bpm by estimating the oxygen saturation percentage and the pulse rate of an artery, equal to the heart rate (Schmitt et al., 1998). The objective of our study was to determine the most effective measurement tool of the above listed based on performance within a production setting.

MATERIALS AND METHODS

The experiment was performed on 18 commercial broiler chickens reared and handled following the Chicken Farmers of Canada Animal Care Program (2018). The birds averaged 2.22 kg and 35 d of age, coming from 3 farms travelling an average of 1.5 h and held at the processing plant for an average of 4.8 h prestun. The sample consisted of sensible (n = 2) birds prior to gas exposure and stunned (n = 10) and confirmed DOA (n = 6) birds after gas exposure. Birds were considered DOA when exiting the gas chamber if exhibiting stiffness as a sign of rigor mortis. Stunned and DOA birds were passed through a 5-chamber Marel Stork Poultry Processing B.V. CAS SmoothFlow conveyor system. Birds were kept in containers holding an average of 70 birds, spending approximately 1 min through each chamber. Chambers 1 to 5 contained average carbon dioxide concentrations of 13.6%, 21.9%, 34.7%, 62.5%, and 72.2%, respectively. Testing started immediately following gas stunning and the combination of all tests did not exceed 5 min for each bird.

Reflexes

Reflex testing was performed on all broiler chickens. Individually, stunned and DOA birds were taken from containers after gas stunning to an antivibration platform adjacent to the stunner exit. They were placed breast-down and tested within the first 60 s of exit for the pupillary light reflex, nictitating membrane reflex and pedal reflex. Sensible birds were tested in lairage, secured under the arm of the examiner to restrict movement without injury.

The pupillary light reflex was performed on stunned and DOA birds by gently opening the eyelid and visually examining the pupil size. It was then shaded from any lights for 5 s before a small light was shone into the eye and the pupil examined again. Sensible birds were examined in dimly lit areas of lairage to get an accurate reaction with the added light. Of the 18 birds, 17 were tested for the nictitating membrane reflex (1 DOA bird was left untested due to membrane injury). In stunned and DOA birds, if the nictitating membrane was open, the inner corner of the eye was gently touched to see if a response was evoked. If no response occurred, a second attempt was done by gently blowing air into the eye. In sensible birds, the eyes were monitored to confirm if the nictitating membrane was how the bird chose to blink when being handled (pseudo blink).

The pedal reflex was performed by placing pressure on the footpad and the toes by pressing between the thumb and index finger to see if a retraction or movement occurred. Sensible birds respond to pressure by either retracting the foot or closing the toes in a grasp. All 18 birds were tested for this reflex.

Doppler Ultrasound

Doppler ultrasound heartbeat monitoring was performed on birds using a ToronTek Pocket Fetal Doppler model R88. Sensible birds were held under the examiner's arm having both wings held to their side to limit movement. The probe was placed under the ribcage aiming up towards the heart. The stunned and DOA birds were individually examined immediately following reflex testing. For this, birds were positioned breast-up on a table. The ultrasound probe was placed under the ribcage and angled upwards toward the heart to avoid the keel bone. Both the left and right sides were tested.

Stethoscopes

Immediately following ultrasound examination, stethoscope auscultation was individually done by placing the bell in the same position as the ultrasound probe. The Littmann Classic III Stethoscope was used for classic stethoscope evaluation and the Littmann 3200 Bluetooth Electronic Stethoscope was used for electronic stethoscope evaluation.

Pulse Oximeter

The DRE Veterinary Avante Waveline Nano-V2 Pulse Oximeter device was used with a clip sensor to collect bpm values. Due to equipment availability, 13 of the 18 test birds (6 stunned, 5 DOA and 2 sensible birds) were tested using the pulse oximeter individually, immediately following stethoscope testing. The device was set to the highest range setting to accommodate poultry bpm range. The clip was placed on a clean toe for all birds. Alternate locations attempted include the wing above the shoulder joint, the leg along the hock and the neck. Results from the toes are reported due to the most consistent detection compared to other locations on the body. Once the clip sensor was in place, the device was left until the value stabilized, and the minimum and

maximum values displayed during this time were recorded.

Statistical Analysis

The doppler ultrasound and stethoscopes did not provide valid bpm readings, resulting in the exclusion of these devices from statistical analysis. Therefore, only data from the pulse oximeter, consisting of differences in bpm between states of consciousness, was analyzed using a generalized linear mixed model (**GLIMMIX**) in SAS, version 9.4 (SAS Institute Inc., Cary, NC). Data were analyzed as a complete randomized design, with each bird considered as an experimental unit. Differences in bpm between DOA, stunned and sensible birds were compared using contrast statements with the state of consciousness and bpm considered the explanatory and response variables, respectively. Significant differences were set at $P < 0.05$.

RESULTS AND DISCUSSION

Reflexes did not aid in identifying a heart rate yet gave an idea to the effectiveness of stunning method (Table 1). As expected, only sensible birds displayed pupil dilation, responsive nictating membranes and pedal reflex responses confirming the effectiveness of the stunning procedure (Erasmus et al., 2010). CAS systems bring broilers into a state of unconsciousness and as such, would be expected to abolish reflex responses. This advocates a lack of pain response or incapability to feel pain during the slaughter process. However, because reflexes do not give insight to a heart rate, they are not beneficial as a sole method of determining life in a bird for the halal community (Fuseini et al. 2019). When paired alongside a tool which can successfully detect heartbeat, reflexes can be used to either confirm death (lack of heartbeat and lack of reflexes) or reaffirm life (presence of heartbeat and lack of reflexes) in a stunned animal.

The doppler ultrasound did not provide any numerical values of bpm when tested on stunned, DOA and sensible birds in the production area. The device was further tested in a noise-reduction booth with no other improvements to results. Chickens have a system of internal air sacs throughout the body which move with the lungs during respiration and can cause difficulty in obtaining a clear pathway between the probe and the heart, alongside the keel bone (Strunk and Wilson, 2003). Without the experience of a trained ultrasound technician to limit interference from bird anatomy and production equipment, even simple ultrasound models can be a difficult tool to manipulate in birds.

Table 2. Values of beats per minute (bpm) between states of consciousness generated for each bird using the pulse oximeter pulse rate (LS-means ± SEM).

States of Consciousness	Average bpm	Minimum and maximum bpm
Sensible (n = 2)	78.3 ± 8.18[a]	20 to 121
Dead on Arrival (DOA, n = 5)	0.0 ± 0.00[b]	0[1]
Stunned (n = 6)	74.8 ± 4.73[a]	24 to 159

[a,b]Different superscripts represent differences in bpm between states of consciousness ($P < 0.05$).
[1]bpm was not detected in any DOA birds evaluated.

Neither stethoscope model provided an audible result which could be concluded as a heartbeat. These methods were further tested in a noise-reduction booth, where the classic model had no improvement. The electronic stethoscope brought more amplification to internal audio, however, was still not strong enough to conclusively determine the heartbeat from other abdominal noises picked up by the device. Using a classic stethoscope is often recommended for avian heartbeat auscultation to help amplify heart sounds around the keel bone (Smith et al., 2014). However, the production setting proves more difficult due to the ambient noise compared to a standard veterinary setting.

The pulse oximeter was the only device able to generate bpm values for all sensible and stunned birds (Table 2). Although the values of bpm did not differ between sensible and stunned birds ($P = 0.724$), DOA birds had a lower bpm than all others ($P < 0.001$). As expected, no bpm (bpm = 0) was detected in all DOA birds. Movement by birds during testing as well as areas with heavy vibration may influence the reading ability of pulse oximeters (Schmitt et al., 1998). However, gentle restraint of sensible birds and the use of a vibration reduction table for stunned and DOA birds successfully decreased interference and provided numerical values to broiler heartbeat in our study. The low statistical power for the comparison between sensible and stunned ($1 - \beta = 0.065$) is a result of the small sample size. Nevertheless, the pulse oximeter was the only device able to distinguish DOA birds from both sensible and stunned birds.

Although the accuracy of precise bpm values deserves further investigation and the small sample size of this

Table 1. Percentage of birds displaying each reflex response to stimuli by states of consciousness.[1]

States of consciousness	Pupillary light reflex response (%)	Nictitating membrane response (%)	Pedal reflex response (%)
Sensible (n = 2)	100	100	100
Dead on Arrival (DOA, n = 6)	0	0[2]	0
Stunned (n = 10)	0	0	0

[1]Descriptive statistics is provided as sensible birds are the only group which displayed reflex responses.
[2]A single untested bird from the total group population.

study posed challenges to our statistical analyses, the achieved results suggest that the pulse oximeter was able to consistently provide bpm values for both stunned and sensible birds in commercial production settings, therefore meeting the requirements for a reliable and practical method in detecting signs of life in broilers. Further studies on this topic are highly encouraged.

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DISCLOSURES

The authors declare no conflicts of interest.

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