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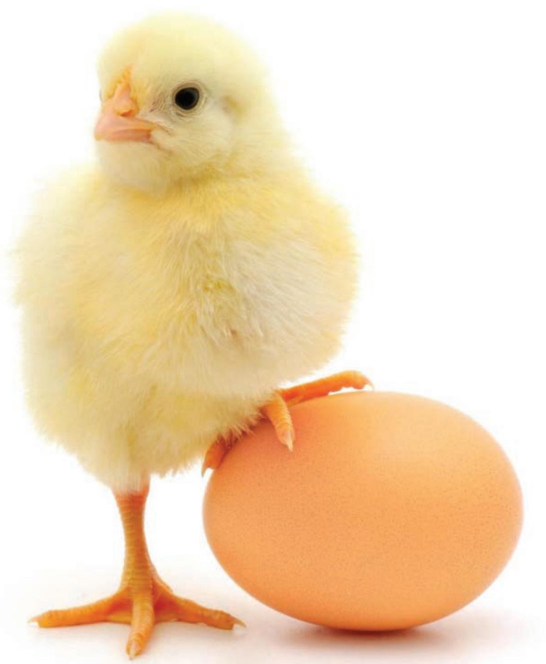

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Research Article
Effect of Light Color on Gas Euthanasia in Neonatal Chickens During Shackling and Corticosterone Levels in Ducks Prior to Slaughter

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Abstract

Background and Objective: A study was conducted to compare euthanizing neonatal birds with carbon dioxide or nitrogen gas in conjunction with various monochromatic light conditions. A second study investigated the effects of monochromatic lighting on corticosterone concentrations prior to stunning in market age birds. **Materials and Methods:** Neonatal male layer chicks, broiler chicks and ducklings were used for gas euthanasia study and market age broiler chickens and Pekin ducks were used for pre-stunning study. Neonates were euthanized using carbon dioxide (CO_2) or Nitrogen (N), while under one of the following lighting conditions, green (G), blue (B), red (R), white (W), or absence of light (Dark). **Results:** All neonates euthanized using N took longer ($p<0.05$) to lose posture than those euthanized with CO_2. Cessation of movement was greater ($p<0.05$) in neonates euthanized with N_2 compared to CO_2. Greater vocalizations ($p<0.05$) were observed in neonates euthanized with N_2 compared to CO_2. While light color did not show differences in vocalizations produced with male layer and broiler chicks, the most vocalizations occurred under the absence of light in ducklings compared to all other colors ($p<0.05$). Light color also showed differences in loss of posture and cessation of movement ($p<0.05$), however there was no consistency between species of neonates. Broiler chickens and Pekin ducks resulted in the highest concentration ($p<0.05$) of corticosterone under Dark, with G resulting in the lowest concentration. **Conclusion:** CO_2 is considered a better means of gaseous euthanasia of neonates compared to N_2. It may be beneficial and recommended to provide G lighting for poultry pre-stunning, however light color should be considered based on the species of poultry and their ability to perceive different wavelengths.

Key words: Light; euthanasia, neonates, stress, carbon dioxide

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Visions is considered the dominant sense of poultry. Birds contain four types of cones, while mammals only contain 3 types[1]. The extra cone within the eye also contains special oil droplets which gives the birds the ability to see partial ultraviolet wavelengths[1]. No research has been done to determine how lighting conditions during euthanasia may affect bird welfare and stress. Two time points where lighting could affect the welfare of poultry during euthanasia are: Prior to slaughter while being shackled prior to stunning and during gas euthanasia at the hatchery of cull or non-desirable chicks. Current practices for pre-slaughter of poultry consist of collection, transport ending with shackling the birds in a dimly lit room[2]. Reduced lighting within the shackling room has been considered to have a calming effect where birds will exhibit a subdued behavior and blue light has been demonstrated to reduce struggling during shackling of broilers[3]. However, no research has been done on the wavelength of light in these rooms and its effect on bird stress. There has been a correlation between the bird's ability to see its environment and the resulting stress and fear. Jones[4] reported that hooded birds resulted in lower instances of vocalizations, wing flapping and signs of struggling when shackled compared to unhooded birds. Previous research has demonstrated that light spectrum can impact stress hormone concentrations and fear in poultry[5-11]. There has been no investigation into stress hormone levels in relation to the light color in the shackling rooms prior to stunning.

Most states have specified methods for humane slaughter of market age farm animals, however male layer chicks are not consumed by humans and therefore have little regulations of welfare-based euthanasia. Male culls are euthanized via maceration or via gaseous euthanasia. According to the AVMA[12], the use of carbon dioxide is an acceptable method of gaseous euthanasia. Carbon dioxide inhalation causes relatively quick insensibilities and death via anoxia[12], however, it can take longer in neonatal birds compared to older birds. Therefore, there is a period of time between initial exposure to the gas and insensibility that may result in distress[13,14]. Furthermore, carbon dioxide gas is highly acidic and forms carbonic acid when in contact with mucosal tissue, which likely causes pain and discomfort[15,16]. As a result of this other inert gaseous such as nitrogen could be an alternative. When using nitrogen for euthanasia, the oxygen must be displaced to less than 2% to create anoxic conditions[12,17]. Nitrogen has not shown to be aversive to chickens or turkeys, while hypoxia is also shown to have little aversive affects[12]. Typically, neonates are euthanized commercially in solid sided boxes resulting complete darkness for the neonates during the process. It is most likely because it is aversive for the workers to watch the euthanasia. This is converse to research settings which require a clear container to be able to observe the effective completion of the euthanasia. These conditions allow light to enter the compartment while the neonates are euthanized in contrast to commercial conditions.

There has been no research on the effect of lighting spectrum on efficacy during euthanasia of neonatal or on the stress during shackling prior to stunning of market age poultry. Therefore, two studies were conducted to investigate these factors. The aim of the first study was to determine if lighting spectrum effected the efficacy of two gaseous euthanasia methods in neonatal ducks and chickens. The second study's aim was to determine if lighting conditions prior to stunning and slaughter affected the stress hormone levels of market age broiler chickens and Pekin ducks.

MATERIALS AND METHODS

Experiment 1: Neonatal euthanasia: Two gaseous methods of euthanasia and pre-slaughter stress under five separate lighting conditions were evaluated and compared in this study. Experimental treatments included Carbon dioxide (CO_2)+white light, CO_2+red light, CO_2+green light, CO_2+blue light, CO_2+absence of light, Nitrogen (N_2)+white light, N_2+red light, N_2+green light, N_2+blue light, N_2+absence of light. Neonates in this study were day-of-hatch Hyline W-36 male layer chicks, day-of-hatch male broiler chicks and day-of-hatch Pekin ducklings. Neonatal euthanasia consisted of 10 replicate trials on male layers, 10 replicate trials on broiler chicks and 5 replicate trials on ducklings. A total of 10 neonates were used in each treatment group for a total of 100 neonates per trial. Each batch of neonates were contained in a box that maintained a comfortable temperature prior to euthanasia and the experimental euthanasia box was cleaned between chicks. Order of treatment application was randomized between trials. Spectrum of each lighting treatment is presented in Fig. 1 as measured by a handheld spectrometer (SFIM-300, Everfine, Hangzhou, China). Light intensities were set to 10 cLux at bird level by using alight meter (Hato Gallilux Meter, Hato Lighting, Sittard, Netherlands). All neonatal ducks and chickens were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching[18] guidelines. All experimental methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP No. 2018-0136).

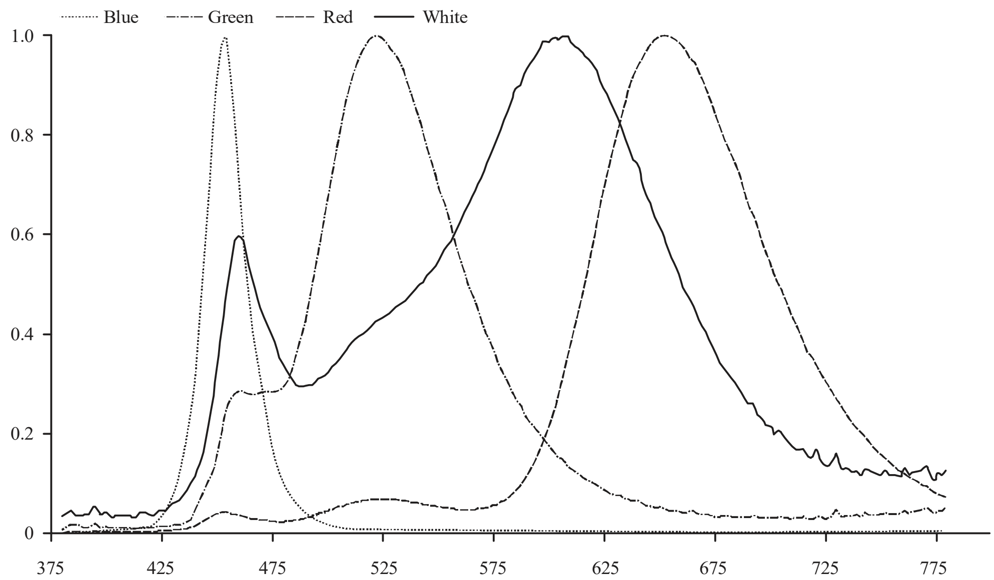

Fig. 1: Spectral power readings of blue, red, green and white experimental prototype LED light fixtures using a spectral flickering irradiance meter

Four treatment groups received exposure to one of the four LED light fixtures, (a) Spectral power readings for blue led light fixture, (b) Spectral power readings for green led light fixture, (c) Spectral power readings for red led light fixture and (d) Spectral power readings for white led light fixture

Neonatal euthanasia procedure: A specialized gas chamber was purchased from Kent Scientific (Torrington, CT, USA). The gas chamber contained a gas inlet and outlet measuring 15 mm length, with the chamber measuring 22.9 cm width, 15.2 cm height, 15.2 cm in diameter with internal volume of 5.29 L. The gas chamber was used for all 10 treatments and chambers were cleaned between birds.

A group of 10 neonates were exposed to each gas treatment and lighting condition until the cessation of movement. Prior to gassing, each neonate was exposed to the lighting treatment for 10 sec inside the chamber. The neonates were held in the gas chamber for one additional minute to ensure death. Flow rates for both gasses were set to 16 Lpm, filling the gas chamber with 100% CO_2 or N_2 after 19.84 sec. Chicks were held for an additional 5 min after cessation of movement and observed for recovery.

Behavioral observations: Each treatment was videotaped to evaluate the behavioral responses of the neonates. The measured variables included the cessation of movement, latency to loss of posture and vocalizations. Each vocalization by a neonate was counted by a tally clicker until cessation of movement. Each neonate in all treatments presented loss of posture and cessation of movement. The loss of posture was determined when the neonates could not stand upright and have control over the body posture[19]. Neonates were determined to have cessation of movement when convulsions such as leg paddling, muscle twitching, wing flapping and all other visible signs of motion completely ceased[19]. Cessation of movement and latency to loss of posture were recorded as time durations, vocalizations were recorded numerically and signs of ataxia (failure to maintain body balance, flipping and gasping for air) were noticed however were not quantified. Neonates under the absence of light treatments were observed using an infrared camera.

Experiment 2: Market age poultry: Treatments consisted of white light, red light, green light, blue light and the absence of light. The light spectrums are presented in Fig. 1 as measured by a handheld spectrometer (SFIM-300, Everfine, Hangzhou, China). Light intensities were set to 10 cLux at bird level by using a light meter (Hato Gallilux Meter, Hato Lighting, Sittard, Netherlands). Broiler chickens (42 days of age) and Pekin ducks (35 days of age) were used for this experiment. Ten trials using 10 broiler chickens per treatment and 5 trials using 10 Pekin ducks per treatment were conducted.

Ten birds were randomly selected and gently shackled in a light tight room. Each treatment of birds was then subjected to either red, blue, green, white or absence of light within the room. All birds were shackled and exposed to the light treatment for a total of 10 min (2 min of which was spent shackled). Each bird was electrically stunned and exsanguinated via neck cutting, trunk blood was then collected in lithium heparin vacutainers. Vacutainers were stored in an ice bath while remaining blood samples were collected and then centrifuged (Centrifuge 5804, Eppendorf, Hamburg, Germany) at 4000 RPM for 15 min. The plasma layer was then poured off into a labeled 2 mL microcentrifuge tube and stored at -20°C until analysis. Samples were thawed at 4°C overnight before analysis. Plasma CORT concentration was obtained using a 96-well commercial ELISA kit (ADI-901-097, Enzo Life Sciences, Inc., Farmingdale, NY), absorbance was read at 450 nm using a microplate absorbance reader (Tecan Sunrise, Tecan Trading AG, Switzerland) and analyzed using the Magellan Tracker software program.

Statistical analysis: All neonatal data was analyzed using GLM procedure with a model of y = trial, light, gas, gas×trial, light×trial, gas×light and gas×light×trial interaction. Trial was found to be nonsignificant ($p>0.05$) and was removed from the model and data was combined and analyzed using the GLM procedure testing the model y = light, gas and light×gas interaction. Similarly, the market age data was analyzed initially with trial in the model but it was removed as it was non-significant, the combined data was analyzed using the ANOVA procedure with the model y = light. Means determined to be significant was separated further by Fishers LSD Test, with accepted significance of $p<0.05$.

RESULTS

Experiment 1: Neonatal euthanasia

Male layer euthanasia: All neonatal male layer culls were successfully euthanized using both gases under all five light treatments data (Table 1). Chicks exposed to N_2 had a longer latency than CO_2 ($p<0.05$) to loss of posture. Differences were seen between colors with red and green light showing longer latencies than all other colors, the shortest latency to loss of posture was observed with the absence of light ($p<0.05$). Nitrogen×red light had the longest latency to loss of posture compared to all other treatments.

Additionally, differences were seen in cessation of movement between gas types, colors and their interaction ($p<0.05$). Nitrogen gas showed to have the longest latency to cessation of movement in the birds compared to CO_2 ($p<0.05$). When comparing light colors, the longest latency to cessation was observed in birds with the absence of light, while the shortest latencies were observed in white and blue light ($p<0.05$). The interaction of gas and light color showed the longest latency with N_2×absence of light, with the shortest latency was observed in CO_2×white light ($p<0.05$).

The latency difference between loss of posture and death showed differences in gas type, light color and their interaction ($p<0.05$). Nitrogen showed to have the longest latency between loss of posture and death compared to CO_2

Table 1: All parameters following euthanasia of neonatal male layer chicks exposed to gas and LED light combinations

Treatments	Loss of posture (sec)	Cessation of movement (sec)	Vocalizations (No. of instances)	Loss of posture to death (sec)	V-Time (vocals/death)[1]
Carbon dioxide (CO_2)	23.67^{a}	78.14^{a}	7.23^{a}	54.48^{a}	0.083^{a}
Nitrogen (N_2)	55.41^{b}	146.01^{b}	99.09^{b}	90.59^{b}	0.673^{b}
Red (580-780 λ)[2]	40.87^{a}	111.7^{bc}	57.27	70.84^{bc}	0.297
Blue (430-480 λ)[2]	39.81^{ab}	109.34^{c}	60.40	69.54^{bc}	0.340
Green (480-580 λ)[2]	40.50^{a}	113.72^{b}	58.63	73.22^{b}	0.303
White (430-780 λ)[2]	39.00^{b}	108.19^{c}	58.14	69.19^{c}	0.445
Dark	37.52^{c}	117.43^{a}	58.96	79.91^{a}	0.246
CO_2×red	23.49^{e}	79.72^{d}	7.44	56.23^{d}	0.094
CO_2×blue	23.79^{e}	78.14^{de}	7.67	54.35^{de}	0.096
CO_2×green	24.07^{e}	79.09^{de}	6.93	55.02^{de}	0.090
CO_2×white	23.83^{e}	74.46^{e}	8.28	50.63^{e}	0.144
CO_2×dark	23.14^{e}	79.30^{de}	5.81	56.16^{d}	0.072
N_2×red	58.24^{a}	143.68^{bc}	97.06	85.44^{c}	0.686
N_2×blue	55.82^{bc}	140.54^{c}	101.29	84.72^{c}	0.732
N_2×green	56.93^{ab}	148.34^{b}	102.11	91.41^{b}	0.697
N_2×white	54.17^{c}	141.93^{c}	97.21	87.76^{bc}	0.698
N_2×dark	51.90^{d}	155.57^{a}	97.76	103.67^{a}	0.653
Pooled SEM	0.64	1.19	1.55	0.56	0.011
p-value gas main effect	p = 0.00	p = 0.00	p = 0.00	p = 0.00	p = 0.00
p-value color main effect	p = 0.00	p = 0.00	p = 0.272	p = 0.00	p = 0.197
p-value Interaction	p = 0.00	p = 0.00	p = 0.225	p = 0.00	p = 0.197

$^{a-e}$Differing superscripts within column indicate significant differences $p<0.05$, [1]V-Time was defined as the ratio of vocalizations until death and [2]Indicates wavelengths of each light color

($p<0.05$). The absence of light was observed to have the longest latency of all colors and the shortest latency was seen in white light ($p<0.05$). When comparing the interaction, N_2×white light showed to have the longest latency compared to all other interactions and the shortest latency was found in CO_2×white light ($p<0.05$).

There were differences in vocalizations between gas type, where the highest instances were observed with N_2, followed by CO_2 ($p<0.05$). There were no differences ($p>0.05$) in number of vocalizations between light color and gas×light color interaction. Additionally, differences in the ratio of vocalizations to cessation of movement (V-Time) were observed between gas type ($p>0.05$). N_2 was observed to have more vocalizations per second compared to CO_2 ($p<0.05$). There were no differences found in V-Time between light color and gas×light color interaction ($p>0.05$).

Broiler chick euthanasia: All neonatal broiler chicks were successfully euthanized using both gas types under all lighting treatments. Table 2 shows the correlation of both gasses along with each light and the interaction of gas and light. For loss of posture, chicks exposed to N_2 had a longer latency than CO_2 ($p<0.05$). Loss of posture showed to have the longest latency under red, white and green light and the shortest latency was observed under blue and the absence of light ($p<0.05$). When comparing the interaction of gas×light color, the longest latency was seen under N_2×red light, N_2×green and N_2×white light, while the shortest latency to cessation was observed in all CO_2×light color treatments ($p<0.05$).

Differences were seen in the latency to cessation of movement in gas type and light color. The N_2 maintained the longest latency compared to CO_2 ($p<0.05$). The longest latencies to cessation between color treatments were observed in green and absence of light ($p<0.05$). There were no differences observed between the interaction of gas type×light color ($p>0.05$).

Differences were observed in latency of loss of posture to death between gas type and color of light. N_2 resulted in longer latency compared to CO_2 ($p<0.05$). Green and the absence of light resulted in the longest latency compared to all other light colors ($p<0.05$).

When comparing vocalizations and the ratio of vocalization (V-Time) to loss of poster, differences were seen with N_2 gas resulting in more instances of vocalization and higher V-Time compared to CO_2 ($p<0.05$). No differences in vocalization and ratio of vocalization to cessation of movement was seen between light color and the interaction of gas type×light color ($p>0.05$).

Duckling euthanasia: All neonatal ducklings were successfully euthanized, results are listed in Table 3. When referring to the latency to loss of posture, CO_2 resulted in a faster latency compared to N_2 ($p<0.05$). The absence of light resulted in the

Table 2: All parameters following euthanasia of neonatal broiler chicks exposed to gas and LED light combinations

Treatments	Loss of posture (sec)	Cessation of movement (sec)	Vocalizations (No. of instances)	Loss of posture to death (sec)	V-Time (vocals/death)[1]
CO_2	24.21[a]	76.80[a]	5.17[a]	52.59[a]	0.053[a]
Nitrogen	57.92[b]	155.85[b]	129.78[b]	97.93[b]	0.629[b]
Red (~580-780 λ)[2]	42.58[a]	114.66[b]	55.73	72.08[b]	0.257
Blue (~430-480 λ)[2]	38.99[b]	112.51[b]	52.44	73.52[b]	0.303
Green (~480-580 λ)[2]	42.48[a]	120.83[a]	47.88	78.35[a]	0.141
White (~430-780 λ)[2]	41.83[a]	113.78[b]	53.80	71.95[bc]	0.291
Dark	39.47[b]	119.81[a]	51.81	80.40[a]	0.148
CO_2×red	23.25[cd]	75.06	5.16	51.81	0.070
CO_2×blue	24.09[cd]	74.38	5.69	50.29	0.078
CO_2×green	23.53[cd]	79.46	5.52	55.93	0.073
CO_2×white	24.66[cd]	75.79	5.66	51.13	0.076
CO_2×dark	25.54[c]	79.31	3.81	53.77	0.048
N_2×red	61.91[a]	154.26	177.26	92.35	1.278
N_2×blue	53.89[b]	150.63	185.18	96.74	1.297
N_2×green	61.43[a]	162.19	84.37	100.76	0.528
N_2×white	58.99[a]	151.76	101.94	92.77	0.689
N_2×dark	53.39[b]	160.42	99.8	107.13	0.629
Pooled SEM	0.64	1.40	1.59	1.03	0.081
p-value for gas main effect	p = 0.00	p = 0.00	p = 0.00	p = 0.00	p = 0.00
p-value for color main effect	p = 0.00	p = 0.00	p = 0.46	p = 0.00	p = 0.10
p-value for interaction	p = 0.00	p = 0.34	p = 0.47	p = 0.05	p = 0.45

[a-e]Differing superscripts within column indicate significant differences ($p<0.05$), [1]V-Time was defined as the ratio of vocalizations until death and [2]Indicates wavelengths of each light color

Table 3: All parameters following euthanasia of neonatal Pekin ducklings exposed to gas and LED light combinations

Treatments	Loss of posture (sec)	Cessation of movement (sec)	Vocalizations (No. of instances)	Loss of posture to death (sec)	V-Time (vocals/death)[1]
CO_2	20.42[a]	92.35[a]	6.29[a]	71.93	0.069[b]
Nitrogen	52.15[b]	123.19[b]	83.56[b]	71.04	0.685[a]
Red (~580-780 λ)[2]	35.98[a]	101.83[d]	33.76[d]	65.85[c]	0.216[c]
Blue (~430-480 λ)[2]	37.19[a]	108.49[b]	47.10[b]	71.30[b]	0.266[ab]
Green (~480-580 λ)[2]	37.26[a]	105.67[c]	46.76[b]	68.41[c]	0.340[a]
White (~430-780 λ)[2]	36.5[a]	115.2[a]	43.93[c]	78.70[a]	0.223[bc]
Dark	34.5[b]	107.67[bc]	53.08[a]	73.17[b]	0.356[a]
CO_2×red	21.26[de]	85.48[e]	3.02[g]	64.22[e]	0.035
CO_2×blue	19.46[ef]	88.92[e]	6.88[ef]	69.46[cd]	0.079
CO_2×green	19.34[f]	91.40[e]	9.04[e]	72.06[c]	0.099
CO_2×white	21.36[d]	105.78[d]	4.32[fg]	84.42[a]	0.041
CO_2×dark	20.68[def]	90.16[e]	8.22[e]	69.48[cd]	0.091
N_2×red	50.70[b]	118.18[c]	64.50[d]	67.48[de]	0.549
N_2×blue	54.92[a]	128.06[a]	87.32[b]	73.14[bc]	0.686
N_2×green	55.18[a]	119.94[c]	84.48[bc]	64.76[e]	0.710
N_2×white	51.64[b]	124.62[b]	83.54[c]	72.98[c]	0.674
N_2×dark	48.32[c]	125.18[ab]	97.94[a]	76.86[b]	0.788
Pooled SEM	0.745	0.829	1.799	0.499	0.014
p-value for gas main effect	p = 0.00	p = 0.00	p = 0.00	p = 0.308	p = 0
p-value for color main effect	p = 0.00	p = 0.00	p = 0.00	p = 0.00	p = 0
p-value for interaction	p = 0.00	p = 0.00	p = 0.00	p = 0.00	p>0.05

[a-g]Differing superscripts within column indicate significant differences ($p<0.05$), [1]V-Time was defined as the ratio of vocalizations until death and [2]Indicates wavelength of each light color

longest latency to loss of posture compared to all other LED light colors ($p<0.05$). The CO_2×Green light resulted in the shortest latency to loss of posture compared to all other gas×light color treatments ($p<0.05$). N_2×Green, N_2×Blue light resulted in the longest latency to loss of posture compared to all gas×light color treatments ($p<0.05$).

The latency to cessation of movement was the longest with N_2 gas compared to CO_2 gas ($p<0.05$). Differences were observed between colors, with the longest latency was seen in white light, with the shortest latency to cessation of movement was seen in red light ($p<0.05$). When referring to the gas type×light color, the shortest latencies were found in CO_2×Red light, CO_2×Blue light, CO_2×Green light and CO_2×absence of light ($p<0.05$). The longest latency to cessation of movement was observed in N_2×Blue light compared to all other gas type×light color treatments ($p<0.05$).

The differences in latency from loss of posture to cessation of movement was not observed between colors and gas type×light color ($p>0.05$). The longest latency was found under white light, while the shortest latency was found under green and red light treatments ($p<0.05$). When comparing gas type×light color, the shortest latency was observed with CO_2×Red light, while the longest latency was observed in CO_2×White light treatments ($p<0.05$).

Instances of vocalizations were the highest under N_2 gas compared to CO_2 gas ($p<0.05$). The highest instance of vocalizations came from the absence of light treatments, with the lowest instance occurred under red light treatments ($p<0.05$). Similarly, the lowest instance of vocalizations occurred under CO_2×Red light compared to all other treatments, while N_2×absence of light had the highest instance of vocalizations ($p<0.05$).

Table 4: Average corticosterone levels from poultry following stunning under different monochromatic lighting

Species	Pekin duck	Broiler chicken
Treatments	Corticosterone (pg mL^{-1})	
Red (~580-780 λ)[1]	18580[b]	19045[ab]
Blue (~430-480 λ)[1]	29440[a]	17504[b]
Green (~480-580 λ)[1]	18491[b]	17799[b]
White (~430-780 λ)[1]	24244[ab]	19645[ab]
Absence of light	30795[a]	21643[a]
Pooled SEM	567	456
p-value	p = 0.00	p = 0.00

[a-e]Differing superscripts within column indicate significant differences ($p<0.05$) and [1]Indicates wavelength of each light color

The ratio of vocalizations to cessation of movement (V-Time) was seen to be the lowest under CO_2 compared to N_2 treatments ($p<0.05$). The highest V-Time was seen in white light treatments, while the lowest V-Time was observed under Green, Blue and absence of light treatments ($p<0.05$). There were no differences observed between the interaction of gas type×light color ($p>0.05$).

Experiment 2: Lighting prior to stunning: All corticosterone levels are presented in Table 4. Differences were observed between light color treatments ($p<0.05$). The absence of light maintained a higher level of corticosterone compared to all other treatments ($p<0.05$). The lowest average levels of corticosterone were the result of blue and green treatments, with white and red treatments were intermediates ($p<0.05$).

DISCUSSION

Experiment 1: Neonatal euthanasia: In this current study, alternative euthanasia lighting and gas methods were examined to determine their efficacy. Nitrogen showed to have longer latencies to loss of posture, latency difference from loss of posture and death and cessation of movement compared to carbon dioxide gas in birds after exposure. This is likely related to the fact that normal atmospheric air consists of 78% N_2 by volume, therefore animals under normal conditions are exposed to a higher concentration of N_2 and as a result a substantially higher concentration is needed to cause loss of consciousness and effectively euthanize animals compared to CO_2. Previous research has demonstrated that the range of 33-36% CO_2 is required to induce unconsciousness in birds, death occurs between 70 and 79% CO_2[20] and a final concentration of 75% CO_2 is sufficient to result in death[17]. Loss of posture and cessation of movement was longer in this current study across all neonates in the N_2 vs the CO_2 supporting the concept that they can withstand much higher levels of N_2 before succumbing to the gas. While the gas is flowing into the chamber, neonates may have been exposed to a lethal amount of CO_2 far quicker compared to N_2[13]. Neonates may have been more susceptible to carbon dioxide at a lower concentration as a result of the anesthetic effect[13]. Similar results have been seen in studies comparing CO_2, N_2 and low atmospheric pressure system (LAPS), where CO_2 gas resulted in the shortest latencies to loss of posture and death[17].

Further making the use of gas to euthanize birds is that not all species respond similarly. The concentration needed to successfully euthanize ducklings may be required to be higher due to the assumption that ducks and other waterfowl are less susceptible to asphyxia and hypoxia[21,22]. Some ducks also contain psychological mechanisms that allow them to withstand hypercapnia by holding their breath, however, the Pekin duck does not dive and belongs to an alternative group known as dabblers[14,15]. The neonatal ducks did appear to lose poster in similar amounts of time under both N_2 and CO_2 when compared to both chicken neonate groups indicating that the ducklings did not have a greater resistance to the gases.

All neonates exposed to carbon dioxide showed to have decreased vocalizations compared to nitrogen gas treatments. Carbon dioxide is known to create a feeling of breathlessness when inhaled[17] and could be one possible reason for the difference in vocalizations between gases. The analgesic effect of CO_2[17] also likely lead to the quicker loss of consciousness across neonates making the total vocalizations less just due to available time to vocalize. While it may be possible that neonates experience greater pain/fear under CO_2 due to the formation of carbonic acid in the mucous membrane and CO_2 gas may have induced the loss consciousness before the carbonic acid was produced, however it is unlikely[23]. The aversion to vocalize is further illustrated in this current study when the ratio of vocalizations to cessation of movement was compared between gases across neonates. The CO_2 resulted in a lower ratio under all neonates, while N_2 resulted in a greater ratio of vocalizations compared to CO_2. If the ratios were equal, it would be expected that willingness to vocalize was equal.

Previously lighting conditions have not been considered during the euthanasia of neonates. The absence of light treatment consistently showed to have the shortest latency to loss of posture across all neonates. White light also had shorter latency to loss of posture in the layer neonates. While the loss of posture would indicate darkness is the best condition to euthanize neonates, other parameters measured in this current study are contraindicative. The latency to cessation of movement and the difference between loss of posture and cessation of movement resulted in mixed results when compared to lighting treatments among neonate types. In both chicken neonate groups the dark condition resulted in the longest time to cessation of movement and time difference from loss of posture to cessation of movement. White light had the shortest of these two measures in both chicken neonate groups. In the ducklings white light had the opposite effect and darkness had an intermediate effect compared to other conditions. These results would seem to indicate that darkness was not as effective in inducing euthanasia as white light in chickens. While in ducks again the opposite appears to be true. However, the loss of posture has been directly correlated to unconsciousness (Insensible) and therefore the light color may not be correlated to latency to cessation of movement and the latency difference between loss of posture and cessation of movement[24]. This may be the result of the neonate's inability to no longer perceive variations of light color as they are insensible. Birds do have pineal and deep brain photoreceptors[25] that would still be stimulated until death and it is unknown if the loss of consciousness would cease any biological responses to that stimulation that may affect the latency to cessation of movement (assumed death). Additionally, when comparing gas type$\times$light color to all parameters, the differences observed were largely the result of the two gas types and not necessary the interactions of gas type$\times$light color.

While the instances of vocalizations resulted in no differences in male layer and broiler culls, the absence of light treatment produced a high number of vocalizations and ducklings produced the most vocalizations. The increased vocalizations could have been the result of the neonates experiencing an increased amount of fear. The restriction in vision has been shown in older broilers to result in increased vocalizations[4], however the neonate chickens did not seem to be more fearful in darkness. However, it is important to note that another species (ducks) did show more fear behavior under darkness during euthanasia.

Experiment 2: Lighting prior to stunning: Lighting is a critical environmental factor for commercially reared poultry and can significantly influence their perceptions of their surroundings. Differences in the temperature of light bulb color and spectrum also impact the fear response of poultry. Archer[6] reported broilers reared under cool LED light (2700 K) had a shorter TI duration and flapped less intensely during INV than broilers under warm LED light (5000 K). Tonic immobility duration was shorter in broilers reared under blue monochromatic light[26] and reduced TI duration has also been reported in ducks reared under monochromatic blue and green LED lighting[26,27]. Poultry perception of light color can also influence their physiological response to stress. Several studies have reported the effects of artificial light color on broilers[5-9] and laying hens[10,11]. Mohamed *et al.*[27] reported elevated plasma CORT in ducks reared under white or red monochromatic LED compared to those exposed to blue or green monochromatic LED light. These previous results indicate that lighting conditions during euthanasia/slaughter could result in differences in stress.

In this current study the corticosterone concentrations were determined in birds shackled under different lighting spectra before stunning. The absence of light resulted in the highest blood concentration in both Pekin ducks and broiler chickens. This may further the argument that under the absence of light birds may experience more fear and stress. While blue light resulted in lower corticosterone concentrations in the broiler chicken in this current study, blue light treatment showed to have high corticosterone concentrations in Peking ducks. Campbell *et al.*[28] observed that ducks reared under blue light had significantly lower body weights and showed to have abnormal behavior with excessive fear[28]. Those results differed from Mohamed *et al.*[27] who indicated that blue light would be the least stressful condition for ducks. Even though ducks and chickens are poultry species but both may have different sensitivity to light spectra[28,29]. It is possible that the blue wavelength used in this current study was more stressful to the ducks than chickens due to visual sensitivity. Ducks in general have a retina that is adapted for a varied environments they inhabit of blue wavelengths[30]. Based on the results of the present study, green or blue light is the least stressful condition for chickens prior to stunning, however, in ducks green or red appears to be the least stressful.

CONCLUSION

Neonates that were subjected to nitrogen took longer to lose posture, cease moving and had higher numbers of vocalizations when compared to all CO_2 treatments. While both nitrogen and carbon dioxide resulted in successful euthanasia, CO_2 should be considered as the more effective means of gaseous euthanasia. Euthanizing neonates in darkness while using gas results in a longer time to death based on the cessation of movement. These results indicate that providing light during gas euthanasia results in a quicker and more effective euthanasia in neonatal poultry. Changes to current commercial euthanasia practices should be considered as darkness is not the optimal lighting condition for this procedure. When comparing light treatments on pre-stunning, while also not practical, the absence of light should not be considered as it resulted in high corticosterone concentrations compared to the other conditions indicating that the birds are under greater distress. Blue light and white light both were observed having high concentrations of plasma corticosterone in Pekin ducks, while blue light had lower concentrations in broiler chickens. It may be beneficial and recommended to provide green lighting for poultry pre-stunning as in both species lower corticosterone concentrations were observed, however light color should be considered based on the species of poultry and their ability to perceive different wavelengths.

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