Interactive Effects of Ammonia and Light Intensity on Ocular, Fear and Leg Health in Broiler Chickens


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Abstract: High atmospheric ammonia is detrimental to poultry health under confined environments, especially in its inducement of eye lesions in juvenile chicks during the first week of grow-out. Furthermore, welfare consultants have expressed concerns that low light-intensity may cause damage to the eye lens or lead to blindness. The objective of the present study was to evaluate the interactive effects of ammonia and light-intensity on ocular, fear and leg health in broiler chickens. The interactive effects of inhalation of ambient air with elevated ammonia concentrations and differing light-intensities on eye lesions and welfare (fear and leg-health) in male and female broiler chickens under environmentally controlled conditions were evaluated. Results indicated that ammonia concentrations at 25 and 50 ppm induced eye lesions after day 7 of initial exposure. Light-intensity alone yielded no significant eye lesions. However, light-intensity of 0.2 and 20 lx for 14 days further exacerbated eye lesions. Nevertheless, the effect of ammonia was more pronounced than that of light-intensity. These conditions worsened as duration of ammonia concentration exposure and light-intensity increased during the 7 days of exposure. Eye lesions induced by the interaction of ammonia and light intensity decreased significantly beginning one week after cessation of ammonia exposure, such that the apparent effects were no longer evident. The findings in this investigation suggest that ammonia induced uveitis in chickens clears rapidly upon cessation of ammonia exposure and that fear along with leg health were not significantly affected by ammonia, light-intensity or their interaction. This suggests that elevated ammonia levels in air and low light intensity did not induce stress in broiler chickens.

Key words: Ammonia, light-intensity, broiler, eye, well-being

Introduction

Atmospheric ammonia has been shown to be detrimental to poultry health and performance (Carlile, 1984; Kristensen and Wathes, 2000; Al Homidan et al., 2003) and is also cited as an environmental concern (NRC, 2003; USDA, 2005). Ammonia production is affected by temperature, moisture and pH of the litter (Elliott and Collins, 1984). Improper ventilation prior to chick placement and during the first week of grow-out often results in elevated ammonia concentration. The adverse effects of ammonia on broiler performance and bird health have been documented in several reviews (Carlile, 1984; Kristensen and Wathes, 2000; Ritz et al., 2004). Ammonia has been shown to cause increased susceptibility to disease and other health-related problems such as Newcastle disease, airsacculitis and keratoconjunctivitis (Bullis et al., 1950; Faddoul and Ringrose, 1950; Anderson et al., 1964). As ammonia increases beyond 40 ppm, broiler performance and carcass quality is adversely affected (Reece et al., 1981; Caveny et al., 1981). However, in a study subjecting a modern genetic strain of broilers to three different concentrations of ammonia, 25 ppm ammonia did not affect 49 day body weight while concentrations of 50 and 75 ppm reduced broiler performance (Miles et al., 2004, 2006). Several workers have shown that respiratory epithelium in birds is damaged by ammonia concentrations in the air exceeding 75 ppm (Al Maghfadani and Beck, 1985; Bottje et al., 1998). Lighting programs that decrease the photoperiod have been shown to minimize skeletal disorders and metabolic diseases (Classen et al., 1991; Renden et al., 1991). Low light intensities have also shown benefits in broiler growth (Charles et al., 1992). However, welfare consultants have expressed concerns that low light intensity may cause damage to the eye lens or lead to blindness (Ashton et al., 1973; Chiu et al., 1975; Cummings et al., 1986; Buyse et al., 1996). Manipulation of normal light perception in birds has been described to be associated with several eye conditions including avian glaucoma, which is induced by prolonged exposure to continuous bright light (Jensen and Matson,
Ammonia addition: For quantitative control of aerial ammonia concentration in the chambers, birds were placed on fresh pine shavings that were 10 cm deep at the beginning of each trial and procedures for ammonia administration were taken that were similar to previous ammonia studies conducted at this laboratory by Miles et al. (2004; 2006). Anhydrous ammonia was continuously metered into six of the chambers to maintain three chambers each at 25 and 50 ppm through panel-mount flowmeters. No ammonia (0 ppm) was added to the remaining three chambers that served as controls.

Ammonia was measured daily at 8:00 AM, 12:00 NOON, 4:00 PM and 8:00 PM during the first 4 days and once a day thereafter through day 14 using a photoacoustic multi-gas-analyzer (INNOVA-1312, Air Tech Instrument, Denmark). Ammonia concentration was measured before and again once or twice after disturbing the chamber atmosphere each day by animal caretakers. During the 14-day exposure period, the average measured ammonia concentration for each treatment level approximated the designated level, but variability with concentration increased because of the association of atmospheric ammonia with that excreted in the shavings. The average concentration for the 25 and 50 ppm treatments were 25.2 ppm and 50.3 ppm, respectively.

Ocular assessments: Eye scoring was performed on days 1, 7, 15 and day 36 by a veterinary ophthalmologist. Also on day 36, the intraocular pressure within the anterior chamber was determined by the veterinary ophthalmologist. In addition, on day 36, five chicks from each chamber were randomly selected and euthanized. The left eyeball was dissected out, weighed, evaluated for gross anatomical anomalies and then prepared for histopathological evaluation by a veterinary pathologist.

Eye examination: At the initiation of each trial, five birds from each chamber were randomly selected for ocular examination. These same numbers of birds were used for subsequent examination through the remainder of the study. The ophthalmologist did not know the treatment origin of any bird examined. Biomicroscopy was performed using a Kowa SL-14 portable slit-lamp (KOWA Company Ltd., Tokyo, Japan). During weekly exams, signs of clinical keratoconjunctivitis and anterior uveitis were recorded. Corneal lesions assessed by biomicroscopy were assigned injury scores similar to Thoft’s classification (Thoft, 1979). The numerical scale for grading corneal lesions was 0 = normal cornea; 0.5 = not normal but less than 1; 1 = diffuse corneal edema generally over greater than three quarters of the corneal surface; 2 = 1 + a focal superficial corneal ulcer measuring less than one quarter of the corneal surface; 3 = 1 + a corneal ulcer of half or more of the corneal surface and extending into the anterior chamber; 4 = 3 +

Materials and Methods

Bird husbandry: In each of the two trials, 792 1-day-old Ross×Ross 708 (Aviagen, Inc., Huntsville, AL) chicks were purchased from a commercial hatchery and randomly distributed into 9 environmentally controlled chambers. There were 44 male and 44 female chicks placed in each chamber. Chicks were vaccinated for Mareks, Newcastle and infectious bronchitis diseases at the hatchery. Each chamber contained fresh pine shavings, tube feeders and a seven nipple watering system. Birds were provided a 3-phase feeding program (starter: 1 to 15 day; grower: 16 to 28 day; finisher: 29 to 36 day). Diets were formulated to meet or exceed NRC (1994) nutrient recommendations. Starter feed was provided as crumbles and subsequent feeds were provided as whole pellets. Feed and water were offered ad libitum. Ambient temperature was maintained at 33°C at the start of experimentation and reduced as the birds progressed in age to ensure comfort, with a final temperature of 21°C at 35 day and thereafter.

Treatments: Treatments consisted of exposure to 0, 25, or 50 ppm of ammonia for 14 days and exposure to 0.2, 2.0, or 20 lx light intensities from day 8 to 36 day of age. Ammonia was metered into 6 of the 9 chambers at 25 or 50 ppm from day 1 to 14 day of age, while the remaining three chambers served as control (0 ppm). Each of the three ammonia level treatments was paired with one of the three light intensity treatments so that each chamber represented a particular ammonia concentration:light - intensity level combination.

Ammonia addition: For quantitative control of aerial macroptalmhos from prolonged exposure to darkness or dim light (Berkovitz et al., 1972; Lauber and Kinnear, 1979). Modern commercial poultry facilities are dimly lit to optimize feed conversion and minimize the incidence of skin scratches associated with higher illuminance and activity. Although the effects of lighting, particularly photoperiod on poultry production are well understood, knowledge of the light intensity on broiler visual abilities and its involvement in the welfare of the bird itself is shallow by comparison. Cummings et al. (1994) reported blindness in pullets exposed to low light intensity (3 lx). The researchers did not measure ammonia concentration, which ammonia content may have confounded the results. The objective of the present study was to evaluate the interactive effects of ammonia and light intensity over time on ocular, Tonic Immobility (TI) and leg health in broiler chickens. It was hypothesized that exposure to elevated ammonia concentrations under different light intensities would adversely affect eye health and the general welfare of broiler chickens.

1957; Lauber and McGinnis, 1966) and avian macromatals from prolonged exposure to darkness or dim light (Berkovitz et al., 1972; Lauber and Kinnear, 1979). Modern commercial poultry facilities are dimly lit to optimize feed conversion and minimize the incidence of skin scratches associated with higher illuminance and activity. Although the effects of lighting, particularly photoperiod on poultry production are well understood, knowledge of the light intensity on broiler visual abilities and its involvement in the welfare of the bird itself is shallow by comparison. Cummings et al. (1994) reported blindness in pullets exposed to low light intensity (3 lx). The researchers did not measure ammonia concentration, which ammonia content may have confounded the results. The objective of the present study was to evaluate the interactive effects of ammonia and light intensity over time on ocular, Tonic Immobility (TI) and leg health in broiler chickens. It was hypothesized that exposure to elevated ammonia concentrations under different light intensities would adversely affect eye health and the general welfare of broiler chickens.

Olanrewaju et al.: Ammonia, Light Intensity, and Ocular Responses
deeper extension into the stromal layers; and 5 = corneal perforation. This scale is also dependent on the
definition of a flare, which is the breakdown of the
blood-eye barrier or protein leakage across this barrier
into the anterior chamber creating cloudiness.
Therefore, the anterior chamber was further assessed
as either 0 = normal anterior chamber; 0.5 = not normal
but less than 1; 1 = flare is visible; 2 = flare is easily
visible; 3 = flare is easily visible with neovascularization
on the iris surface; or 4 = flare is easily visible with
hyphema clearly evident and diffuse iris
neovascularization.

Histopathologic examination: At the end of each trial
day 36), five birds from each treatment were randomly
selected and euthanatized by cervical dislocation for
histopathologic assessment. Tissue samples, including
trachea and the entire head, were placed into 10%
buffered formalin. After fixation was complete, the left eye
was enucleated from each head and placed in a 5%
nitric acid solution for 24 hr to decalcify the scleral
ossicles. Following decalcification, a longitudinal
section was trimmed from each eye through the center
of the cornea and then washed in running water for an
additional 24 hr to remove the acid. Two transverse
sections were trimmed from each trachea: one from the
proximal end and one from the distal end. The eye
specimens and trimmed specimens of trachea were
then processed routinely, embedded in paraffin,
sectioned at 5 µm and stained with hematoxylin and
eosin. The examining pathologist was unaware of bird
treatment origin. The iris and ciliary body were scored for
the presence (+) or absence (-) of heterophils, diffuse
lymphocytic infiltrates and nodular lymphocytic infiltrates.
In addition, the presence (+) or absence (-) of increased
cellularity along the rostral surface of the iris was also
noted and the corneal epithelium was scored for the
presence (+) or absence (-) of ulceration. Tracheas were
scored for lymphocytic and heterophilic infiltrates in each
section. Lymphoid infiltrates were scored as follows: (1)
none or rare; (2) lymphocytic infiltrates but no germinal
center formation; (3) one germinal center seen; and (4)
more than one germinal center seen. Heterophil
infiltrates were scored as follows: (1) none or rare; (2)
small numbers (must use 10X lens to confirm
presence); (3) moderate numbers (easily seen at 4X); and
(4) large numbers (confluent infiltrates).

General well-being: On day 28, 10 chicks from each
chamber were randomly selected for assessment of
their general welfare using three different protocols.
Welfare locomotive ability was assessed using a
modification of the Kestin Gait Scoring System (Kristin et
al., 1994) as described in the American Humane
Welfare Standard. Fear and frustration was assessed by
determining tonic immobility index time (American
Humane Welfare Standard). In addition, unnecessary

Gait Scoring (GS) test: Two chicks at a time were
allowed to walk freely (1.52 m) in an inside enclosed
floor area of 1.83 m×3.66 m that contained fresh pine
shavings. Gait score performance was evaluated
according to the Kestin Gait Scoring System (Kristin et
al., 1994) and modified by Dawkins et al. (2004) on a
scale ranging from 0 to 2. Score 0 represented no
detectable impairment of walking, score 1 indicated
birds with no detectable walking impairment and able to
walk at least 5 feet without sitting down, while score 2
indicated severe impairment of walking ability with birds
being unable to walk 5 feet without sitting down again.
Chicks assigned a score 2 were unable to walk. Each
chick was observed for 2 to 3 min. If the chick hesitated
or remained immobile, it was touched with a long stick
to encourage it to walk.

Tonic Immobility (TI): Tonic immobility was induced by
inverting the bird on its back and restraining it for 10 s in
a U-shaped wooden cradle covered with a layer of cloth.
One hand was used to cover the birds head and the
other hand was placed on the sternum, as described by
Jones and Waddington (1992). Eye contact was
completely avoided between the bird and the
experimenter after the experimenter removed his hands
from the cradle. A stopwatch was used to record
latencies until the bird righted itself (getting to its feet
again). The time was measured from withdrawal of the
hand until the bird straightened up. If the bird righted
itself in less than 10 s, then TI was not considered to
have been induced. If TI was not induced after 3
attempts, the duration of TI was considered to be 0 s
and the restraining procedure had to be repeated. If the
bird did not show a righting response over the 10 s test
period, then a maximum score of 600 was given for
righting time. The number of inductions required to attain
TI was also recorded for each bird.

Statistical analysis: A 3×3 factorial treatment
arrangement in a randomized complete design was
used in this study. The 9 treatments consisted of 3
levels of ammonia concentrations and 3 levels of light
intensities. Main effects of ammonia, light intensity and
their interaction were statistically evaluated by the MIXED
procedure (SAS, 2004). Two trials were repeated over
time where trial served as the blocking factor. Geometric
means are presented (Table 1 and 2) for the corneal
and anterior chamber scores. The histopathologic eye
tissue evaluations (presented as percent of occurrence
in Table 3) required arcsine transformation. Means were
separated using Least Significant Difference (LSD)
comparisons at P ≤ 0.05 (SAS, 2004).
Olanrewaju et al.: Ammonia, Light Intensity, and Ocular Responses

Table 1: Clinical corneal lesion and anterior chamber scores of male broilers exposed to graded levels of ammonia and light intensities on day 7

<table>
<thead>
<tr>
<th>Ammonia addition (ppm)</th>
<th>Light intensity (lx)</th>
<th>Corneal Lesion Score</th>
<th>Anterior Chamber Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>20</td>
<td>0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>20</td>
<td>0.02b&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td>0.049</td>
<td>0.054</td>
</tr>
</tbody>
</table>

Means within column and row are presented on log transformed values. There were 5 observations per mean from each chamber (N=2).

1Numerical scale for grading corneal lesions: 0 = normal cornea, ½ = not normal but < 1, 1 = diffuse corneal edema generally over > 3 /4 of the corneal surface, 2 = 1 + a focal superficial corneal ulcer measuring < ¼ of the corneal surface, 3 = 1 + a corneal ulcer of ½ or more of the corneal surface and extending into the anterior stroma and 4 = 3 + deeper extension into the stromal layers, 5 = corneal perforation;

2Numerical scale for grading anterior chamber: 0 = normal anterior chamber, ½ = not normal but < 1, 1 = flare is visible, 2 = flare is easily visible, 3 = flare is easily visible with neovascularization on the iris surface and 4 = flare is easily visible with hyphema clearly evident and diffuse iris neovascularization;

3Pooled SEM for interaction effect (n = 2);

4Means with a row or column possessing different letter (s) differ by LSD test at p < 0.05.

Results

Eye examination: Effects of the exposure to elevated ammonia concentrations in the presence of 20 lx light-intensity from day 1 to day 7 of exposure on corneal lesion scores and anterior chamber scores of broiler chickens are summarized in Table 1. There was significant (p ≤ 0.05) effect of 50 - ppm ammonia on corneal lesion compared to 25 and 0-ppm ammonia concentrations, respectively. However, there was no significant difference among the treatments on anterior chamber lesion.

Concurrent inhalation of elevated ammonia concentrations along with different light intensities from day 7 to day 14 significantly exacerbated eye lesions.

Table 2: Clinical Corneal Lesion Score (CLS) and Anterior Chamber Score (ACS) of broilers exposed to graded levels of ammonia and light intensities on days 14 and 36

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 14</th>
<th>Day 36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia-intensity</td>
<td>CLS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ACS&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 ppm-0.2 lx</td>
<td>0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 ppm-2.0 lx</td>
<td>0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 ppm-20 lx</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 ppm-0.2 lx</td>
<td>0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 ppm-2.0 lx</td>
<td>0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 ppm-20 lx</td>
<td>0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 ppm-0.2 lx</td>
<td>2.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 ppm-2.0 lx</td>
<td>1.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 ppm-20 lx</td>
<td>1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.263</td>
<td>0.107</td>
</tr>
<tr>
<td>Source of Variation</td>
<td>CLS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ACS&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.0611</td>
<td>0.1715</td>
</tr>
<tr>
<td>Intensity</td>
<td>0.4726</td>
<td>0.7361</td>
</tr>
<tr>
<td>Ammonia×intensity</td>
<td>0.0263</td>
<td>0.0494</td>
</tr>
</tbody>
</table>

1Means within a column and effect that lack common superscripts differ significantly by LSD at P#0.05 on log transformed values. There were 5 observations per mean from each chamber (N = 2);

2Numerical scale for grading corneal lesions: 0 = normal cornea, ½ = not normal but < 1, 1 = diffuse corneal edema generally over > 3 /4 of the corneal surface, 2 = 1 + a focal superficial corneal ulcer measuring < ¼ of the corneal surface, 3 = 1 + a corneal ulcer of ½ or more of the corneal surface and extending into the anterior stroma and 4 = 3 + deeper extension into the stromal layers, 5 = corneal perforation;

3Numerical scale for grading anterior chamber: 0 = normal anterior chamber, ½ = not normal but < 1, 1 = flare is visible, 2 = flare is easily visible, 3 = flare is easily visible with neovascularization on the iris surface and 4 = flare is easily visible with hyphema clearly evident and diffuse iris neovascularization;

4Pooled SEM for interaction effect (n = 2)
Table 3: Influence of ammonia and light-intensity on histological changes noted in the iris, ciliary and trachea (as percentage of occurrence) in broiler chickens at 36 day of age

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Iris</th>
<th>Ciliary Body</th>
<th>Trachea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diffuse</td>
<td>lymphocytic infiltrates</td>
<td>Heterophilic infiltrates</td>
</tr>
<tr>
<td>Ammonia-intensity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ppm-0.2 lx</td>
<td>46.80</td>
<td>48.30</td>
<td>20.10</td>
</tr>
<tr>
<td>0 ppm-2.0 lx</td>
<td>40.50</td>
<td>48.50</td>
<td>20.10</td>
</tr>
<tr>
<td>0 ppm-20 lx</td>
<td>50.90</td>
<td>42.10</td>
<td>22.30</td>
</tr>
<tr>
<td>25 ppm-0.2 lx</td>
<td>79.80</td>
<td>49.70</td>
<td>30.10</td>
</tr>
<tr>
<td>25 ppm-2.0 lx</td>
<td>80.20</td>
<td>50.50</td>
<td>30.30</td>
</tr>
<tr>
<td>25 ppm-20 lx</td>
<td>81.50</td>
<td>54.60</td>
<td>31.20</td>
</tr>
<tr>
<td>50 ppm-0.2 lx</td>
<td>89.07</td>
<td>90.70</td>
<td>56.10</td>
</tr>
<tr>
<td>50 ppm-2.0 lx</td>
<td>85.40</td>
<td>89.40</td>
<td>56.40</td>
</tr>
<tr>
<td>50 ppm-20 lx</td>
<td>84.30</td>
<td>87.30</td>
<td>56.70</td>
</tr>
<tr>
<td>SEM</td>
<td>6.328</td>
<td>6.788</td>
<td>5.1313</td>
</tr>
</tbody>
</table>

Source of Variation | Ammonia | Intensity | Ammonia-intensity

Means within a column and effect that lack common superscripts differ significantly by LSD at P ≤ 0.05 on arcsine transformed values. There were 5 observations per mean from each chamber (N = 2).

Table 4: Influence of ammonia and light-intensity on Tonic Immobility (TI) and gait-score (GS) in broiler chickens at 28 day of age

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TI (s)</th>
<th>GS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia-intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ppm-0.2 lx</td>
<td>177.7</td>
<td>20.60</td>
</tr>
<tr>
<td>0 ppm-2.0 lx</td>
<td>181.4</td>
<td>15.71</td>
</tr>
<tr>
<td>0 ppm-20 lx</td>
<td>191.4</td>
<td>15.40</td>
</tr>
<tr>
<td>25 ppm-0.2 lx</td>
<td>194.7</td>
<td>15.80</td>
</tr>
<tr>
<td>25 ppm-2.0 lx</td>
<td>178.4</td>
<td>15.42</td>
</tr>
<tr>
<td>25 ppm-20 lx</td>
<td>188.2</td>
<td>20.10</td>
</tr>
<tr>
<td>50 ppm-0.2 lx</td>
<td>195.7</td>
<td>20.75</td>
</tr>
<tr>
<td>50 ppm-2.0 lx</td>
<td>192.1</td>
<td>20.20</td>
</tr>
<tr>
<td>50 ppm-20 lx</td>
<td>195.8</td>
<td>20.10</td>
</tr>
<tr>
<td>SEM</td>
<td>6.820</td>
<td>1.363</td>
</tr>
</tbody>
</table>

Source of Variation | Ammonia | Intensity | Ammonia-intensity
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>0.5313</td>
<td>0.6471</td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>0.7472</td>
<td>0.8642</td>
<td></td>
</tr>
<tr>
<td>Ammonia-intensity</td>
<td>0.7933</td>
<td>0.7365</td>
<td></td>
</tr>
</tbody>
</table>

Pooled SEM for interaction effect (n = 2)

Compared to those from day 1 through day 7 (Table 2). On day 14, which was the last day of ammonia exposure, corneal lesions scores and anterior chamber scores increased with increasing concentration of ammonia and lower light intensity. There were interactive effects of ammonia and light intensity on corneal and anterior chamber lesions. These observations were more pronounced under 50-ppm ammonia and 0.2 lx light intensity. The effect of ammonia increasing concentration was more pronounced than that of decreasing light intensity, in that there was no significant effect of light intensity in the absence of ammonia. In addition, Table 2 shows the interactive effects of ammonia and light intensity on eye lesions in broiler chickens on day 36. Three weeks after the end of ammonia exposure while light intensity treatments were still ongoing, eye lesions induced by the interaction of ammonia concentration and light intensity quickly decreased and there was no significant difference among the treatments. This decrease started one week after the first two weeks of ammonia exposure. However, in the absence of an ammonia environment between days 15 and 36, healing occurred, as indicate by the declined in mean corneal lesions and anterior chamber scores for both the 25 and 50 ppm treatments.

Table 3 presents histopathologic examination changes due to the interactive effects of ammonia and light intensity on rostral surface, lymphocytes and heterophils in the iris stroma, ciliary body and trachea of broiler chickens were noted. Statistical significant treatment differences due to ammonia were noted among iris and ciliary body lymphocytic and heterophilic infiltrates.
However, the differences among means were more pronounced at the highest ammonia levels (50-ppm). The iris rostral surface demonstrated treatment trends that were similar to those of the iris and ciliary body lymphocytic and heterophilic infiltrates at all levels of ammonia and light intensity exposure. Histopathologic data for nodular lymphocytic infiltrates in the iris, ciliary and whole conjunctiva tissues were excluded from Table 3 because they were negligible. For the conjunctiva, a certain amount of (nodular) lymphoid tissue and corneal stroma are likely normal.

Table 4 represents results of the interactive effects of ammonia and light intensity on TI and gait scores. The overall GS values were less than 1 and no birds were found to have GS > 2. Tonic immobility and gait scores were not significantly affected by either ammonia or light intensity. In addition, ammonia and light intensity did not significantly interact to TI or gait score.

Discussion
The aim of this study was to determine the interactive effects of ammonia and light intensity on ocular, fear and leg health in broiler chickens. It was hypothesized that exposure to elevated ammonia concentrations under different light intensities would adversely affect eye health and the general welfare of broiler chickens. Results indicated that broilers exposed to both low (25 ppm) and high (50 ppm) levels of ammonia gas experienced ocular changes and the symptoms were exacerbated at high concentrations of ammonia during the 14 days of exposure, regardless of light intensity. Light intensities alone yielded no significant eye lesions. The interaction of ammonia concentration with light-intensities of 0.2 and 20 lx demonstrated exacerbating of eye lesions caused by elevated of ammonia concentrations by these light intensities. These conditions worsened as duration of ammonia concentration exposure increased and light-intensity decreased from day 7 of exposure. The eye lesions induced by the interaction of ammonia concentration and light intensity quickly improved by one week following the end of ammonia exposure while light intensity treatments were still ongoing. The effect of ammonia was more pronounced than that of light intensity. These data indicate that ammonia has the greatest detrimental effect on ocular health of young broilers, although higher or lower light intensities may exacerbate the effects of ammonia. Ammonia induced uveitis in chickens clears rapidly upon cessation of ammonia exposure and there were no effects of ammonia, light-intensity or their interaction on fear and leg-health.

The adverse effects of this exposure are primarily seen in the eyes and respiratory tract. Ammonia is the primary cause of keratoconjunctivitis and tracheitis in chickens (Bullis et al., 1950; Faddoul and Ringrose, 1950; Al Maghhadani and Beck, 1985). An idiopathic ocular disorder in young chicks, designated keratoconjunctivitis, was first described by Bullis et al. (1950), who attributed it to environmental factors in the rearing facilities. Anderson et al. (1964) reported that chickens continuously exposed to ammonia at 20 ppm showed some signs of discomfort, including rubbing of the eyes, slight lachrymation, anorexia and, weight loss. In that study, chickens exposed to 20 ppm over a period of 42 days show pulmonary congestion, edema and hemorrhage. Symptoms of ammonia poisoning in poultry include snicking, tracheal irritation, air sac inflammation, conjunctivitis, keratoconjunctivitis and dyspnea (Kling, 1974; Kling and Quarles, 1974; Carlile, 1984). Ocular changes in the present study included significant corneal ulcerations starting at 7 days of age and the anterior chamber exhibited abnormalities at the same period. These findings were consistent with recent reports by Miles et al. (2004; 2006). However, our data are in contrast to a recent report by Beker et al. (2004), which showed no corneal lesions after 3 wk exposure to 0, 30, or 60 ppm ammonia. Lymphocytes and heterophilic infiltrates in the iris generally followed the same trends observed in the cornea and anterior chamber. Because birds were allowed to recover from the ammonia exposure (the ammonia was discontinued after 14 days of age to mimic brooding period), the corneal tissue from 36 day old broiler chickens demonstrated few pathologic changes across the treatments. The low occurrence of histologic changes in the cornea coincided with ophthalmologic evaluations on day 36. The predominant histologic lesions observed were confined to the iris and ciliary body. Heterophils and lymphocytes were mostly seen across the anterior iris surface with some infiltrate in the tissue stroma.

Low light intensities have been shown to have a positive effect on broiler growth (Charles et al., 1992), but welfare consultants have expressed concerns that low light intensity may cause damage to the eye lens or lead to blindness (Ashton et al., 1973; Chiu et al., 1975; Cummings et al., 1986; Buyse et al., 1996). Commercial broilers are reared in artificial light, which differs from natural light in terms of light intensity, color, photoperiod and flicker. Blindness of pullets exposed to low light intensity (3 lx) has been reported (Cummings et al., 1986), but ammonia concentration was not documented in their study. Therefore, ammonia content may have confounded their results. However, our present results indicated that light intensities alone yielded no significant eye lesions. Ammonia treatments induced eye lesions and the interaction of ammonia concentration on eye lesions.

Tissue from the tracheas did not appear to be negatively affected by any of the treatments. Trachea lymphocytic and heterophilic infiltrates means were not statistically different due to ammonia concentrations, which is in agreement with Miles et al. (2006). In contrast, Valentine (1964) reported tracheitis in chicks exposed to ammonia at 60-70 ppm. Valentine (1964) reported tracheitis in
chicks exposed to ammonia at 60-70 ppm. It was suggested that tracheitis may predispose the affected birds to respiratory diseases with the added risks of secondary infections. Air sacculitis has been associated with high ammonia concentrations in poultry houses (Ernst, 1968), and it has also been experimentally induced in chickens exposed to atmospheric ammonia and the stress of infectious bronchitis vaccination (Kling and Quarles, 1974).

Leg abnormalities and fear in broilers are economic and welfare concern in poultry production. The economic costs associated with leg weakness including culling and condemnations or downgrading at processing plant. However, recent reports indicated that Gait Scores (GS) and the incidence of leg weakness may have improved over the last decade (Classen et al., 2003, 2004). Results of this study showed that both GS and TI were generally normal with no significant differences occurring between treatments. This indicates that intensive selection against skeletal abnormalities has improved the skeletal condition, in agreement with industry awareness and recent reports by Classen et al. (2003, 2004). Furthermore, Ross×Ross 708 broilers were used in this study and leg abnormalities are not typically problematic with this strain of broilers. The duration of TI was similar for all the treatments. Duration of TI has been described as a good predictor of the level of fearfulness in domestic chickens (Jones, 1986). Unlike our present results, the incidence of leg problems has been shown to be influenced by light intensity (Newberry et al., 1988), photoperiod (Wilson et al., 1984) and light color (Prayitno et al., 1997).

A sizable body of scientific literature including Downing and Bryden (1999) and Thaxton (2004) have accumulated concerning stress and the welfare of domestic fowl, especially juvenile birds. However, stress and welfare have not been considered collectively. The results of this study indicate that ammonia concentrations at 25 and 50 ppm induced eye lesions beginning at day 7 of exposure. No significant eye lesions resulted from exposure to differing levels of light intensity. The findings in this investigation suggest that ammonia induced uveitis in chickens clears rapidly upon cessation of ammonia exposure and that ammonia concentrations and light-intensity did not interact or act independently to effect fear or leg-health in broilers suggesting that these factors did not pose as stressors to the birds.

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References


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