Effect of N-nitrosoatrazine on Embryogenesis in Avian Embryos

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Effect of N-nitrosoatrazine on Embryogenesis in Avian Embryos
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Introduction
Nitrates and atrazine are common drinking water contaminants (particularly in agricultural communities) and frequently occur together. N-nitrosoatrazine (NNAT) forms in the acidic environment of the human stomach when nitrite and atrazine are present together. We seek a deeper understanding of how nitrosamines disrupt embryonic development, for which NNAT will serve as a model. NNAT was dissolved in dimethyl sulfoxide (DMSO) for administration to the air sac of the fertilized eggs.

Objectives/Purposes
The objectives of this study were to:
- a) Evaluate the effects of DMSO on the weight and mortality of embryo
- b) Evaluate the effect of NNAT on the weight and mortality of embryo
- c) Determine the LD50 (lethal dose of 50% of a test population) of NNAT on developing embryos

Materials and Methods
- Fertilized chicken eggs were acquired from Nelson Poultry Farms in Manhattan, Kansas.
- The experiment was conducted in eight lots of fertilized eggs incubated at 38°C in a humidified, rocking incubator (Little Giant). Each lot consisted of 42 eggs.
- The eggs were treated at Hamburger and Hamilton (HH) stage 9–10 (7–10 somites), by injecting solution into the air sac above the embryo through a small opening in the shell.
- Embryos were harvested on day 5 of development (HH stage 27), and examined for mortality and weight.
- The analysis was separated into three different experiments to study the objectives above.
- Experiment 1 evaluated the DMSO effect on the weight and mortality of embryos.
- Experiment 2 evaluated the effect of combination of DMSO with water and effect of NNAT at low dose level.
- Experiment 3 evaluated the effect of different doses of NNAT on the weight and mortality of the embryos.

Hypothesis
We hypothesized that chicken embryos exposed to NNAT would have delayed development and increased mortality compared to unexposed embryos.

Design
Lot 1 was analyzed as Completely Randomized Design (CRD). We found that there appeared to be position effects where the eggs near the heat source had higher weight means compared to the eggs further from the heat source.

• Lot 1 was analyzed as Completely Randomized Design (CRD). We found that there appeared to be position effects where the eggs near the heat source had higher weight means compared to the eggs further from the heat source.
• Lot 2 was analyzed as Randomized Complete Block Design and blocked the experimental units into rows. There seemed to be column effects in Lot 2.
• Lot 3 was analyzed as Row Column Design.

Table 1: Experimental Design

<table>
<thead>
<tr>
<th>Lot</th>
<th>Experimental Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CRD – “appeared” to have position effects</td>
</tr>
<tr>
<td>2</td>
<td>RCBD – blocked by rows of six eggs in incubators, “appeared” to have column effects</td>
</tr>
<tr>
<td>3-8</td>
<td>Row Column Design</td>
</tr>
</tbody>
</table>

Results
Table 3: Effect of treatment on weight and mortality

<table>
<thead>
<tr>
<th>Conclusion</th>
<th>Weight</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>No significant differences</td>
<td>p-value=0.0619</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>No significant differences</td>
<td>p-value=0.4288</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>No significant differences</td>
<td>p-value=0.1262</td>
</tr>
</tbody>
</table>

Table 4: Mean weight and Mortality Rates Experiments 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Weight (gram)</th>
<th>MSE</th>
<th>Mortality Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.2493</td>
<td>0.00621</td>
<td>9.75%</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.2012</td>
<td>0.0801</td>
<td>21.05%</td>
</tr>
<tr>
<td>Water</td>
<td>0.2272</td>
<td></td>
<td>21.05%</td>
</tr>
</tbody>
</table>

Table 5: Mean weight and Mortality Rates Experiments 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Weight (gram)</th>
<th>MSE</th>
<th>Mortality Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0.1373</td>
<td>0.00355</td>
<td>21.05%*</td>
</tr>
<tr>
<td>Water/DMSO</td>
<td>0.1633</td>
<td></td>
<td>21.05%*</td>
</tr>
<tr>
<td>NNAT 0.245 in DMSO</td>
<td>0.1449</td>
<td></td>
<td>21.05%*</td>
</tr>
</tbody>
</table>

Table 6: Mean weight and Mortality Rates Experiments 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Weight (gram)</th>
<th>MSE</th>
<th>Mortality Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0.0801</td>
<td>0.0025</td>
<td>25.64%</td>
</tr>
<tr>
<td>NNAT 1.11 in DMSO</td>
<td>0.1218</td>
<td></td>
<td>25.64%</td>
</tr>
<tr>
<td>NNAT 2.22 in DMSO</td>
<td>0.0972</td>
<td></td>
<td>25.64%</td>
</tr>
<tr>
<td>NNAT 3.33 in DMSO</td>
<td>0.0948</td>
<td></td>
<td>25.64%</td>
</tr>
</tbody>
</table>

Figure 1: LD50 plot
Based on linear regression analysis, the LD50 was determined to be 2.85 µmol/l.

Overall Conclusions
- We found that there were no significant differences of treatments on weight for all three experiments.
- In terms of mortality
  - Experiment 1, there were no significant differences of treatments (Water, DMSO, Blank).
  - No conclusion was made for Experiment 2 since for two out of three treatments had zero mortality.
  - For Experiment 3, there was a significant linear increase in mortality as NNAT dose increased. These results showed that the higher the NNAT dose, the higher the mortality but once the eggs survive there were no significant effects of treatments on the development of embryos.

Discussions/Recommendations
- This study investigated the potential for adverse health impacts on chick embryos due to exposure to NNAT.
- Embryo mortality increased linearly with higher levels of NNAT.
- Embryos appeared to have a threshold response to NNAT where if they survived past a certain threshold, their weights were unaffected.

Acknowledgements
- Funding: NU Foundation Research Council
- We thank Dr. Tom Rosenquist for assessing the presence of malformations in the embryos.
- Contact Information: nfrosian@hotmail.com