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Nur Firyal Roslan
University of Nebraska - Lincoln

Moses New-Aaron
University of Nebraska Medical Center, moses.newaaron@unmc.edu

Martha Rhoades
University of Nebraska - Lincoln

Kent Eskridge
University of Nebraska - Lincoln

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Effect of *N*-nitrosoatrazine on Embryogenesis in Avian Embryos

Nur Firyal Roslan¹, Moses New-Aaron², Martha Rhoades¹, Kent Eskridge¹
University of Nebraska-Lincoln¹, University of Nebraska Medical Center²



Introduction

- Nitrate 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.
- The purpose of this research was to gain a deeper understanding of NNAT toxicity to the developing embryo. Understanding how NNAT disrupts embryo development could be paradigm-changing in studying the effects of nitrosamines on human health. Chicken embryos are widely used to study early development because they provide a rapid model for embryotoxicity.

Objectives/Purposes

- The objectives of this study were to:
- Evaluate the effects of DMSO on the weight and mortality of embryo
 - Evaluate the effect of NNAT on the weight and mortality of embryo
 - 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

Design Structure

Table 1: Experimental Design

Lot	Experimental Design
1	CRD – “appeared” to have position effects
2	RCBD – blocked by rows of six eggs in incubators, “appeared” to have column effects
3-8	Row Column 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.
- We then changed Lot 2 design to Randomized Complete Block Design and blocked the experimental units into rows. There seemed to be column effects in Lot 2.
- We then changed the design to row and column design where we blocked the incubator by both directions and used this design for the rest of the lots.

Treatment Structure

Table 2: Treatment Design

Experiment	Lot	Treatments
1	1, 2, 3	Water Blank DMSO
2	4, 8	DMSO 50:50 Water:DMSO NNAT 0.245 in DMSO
3	5, 6, 7	Blank** DMSO NNAT 1.11 in DMSO NNAT 2.22 in DMSO NNAT 3.33 in DMSO

** is control treatments and were not included in the analysis

- Lots with the same treatments were grouped together into three experiments and analyses were done based on weight and mortality of embryos.
- Experiment 1 was analyzed as Combined Experiments over lots 1-3 since different lots had different experimental designs.
- Experiment 2 and Experiment 3 were analyzed as Row and Column designs.

Results

- Table 3 below shows the effect of experiment on weight and mortality.

Table 3: Effect of treatment on weight and mortality

Conclusion	Weight	Mortality
Experiment 1	No significant differences p-value=0.0619	No significant differences p-value=0.1141
Experiment 2	No significant differences p-value=0.4288	Could not make conclusions because of zero mortality for two treatments
Experiment 3	No significant differences p-value=0.1262	Significant linear increase as NNAT dose increased p-value=0.0345

- Table 4-6 below give all the mean weight in gram (assuming the response to be normally distribution) and the mortality rates (assuming the response to be binary distribution) of each treatments.

Table 4: Mean weight and Mortality Rates Experiment 1

Treatment	Blank	DMSO	Water
Mean Weight (gram)	0.2493	0.2012	0.2272
MSE	0.00621		
Mortality Probability	9.75%	25.44%	6.45%
Standard Error	0.068	0.124	0.053

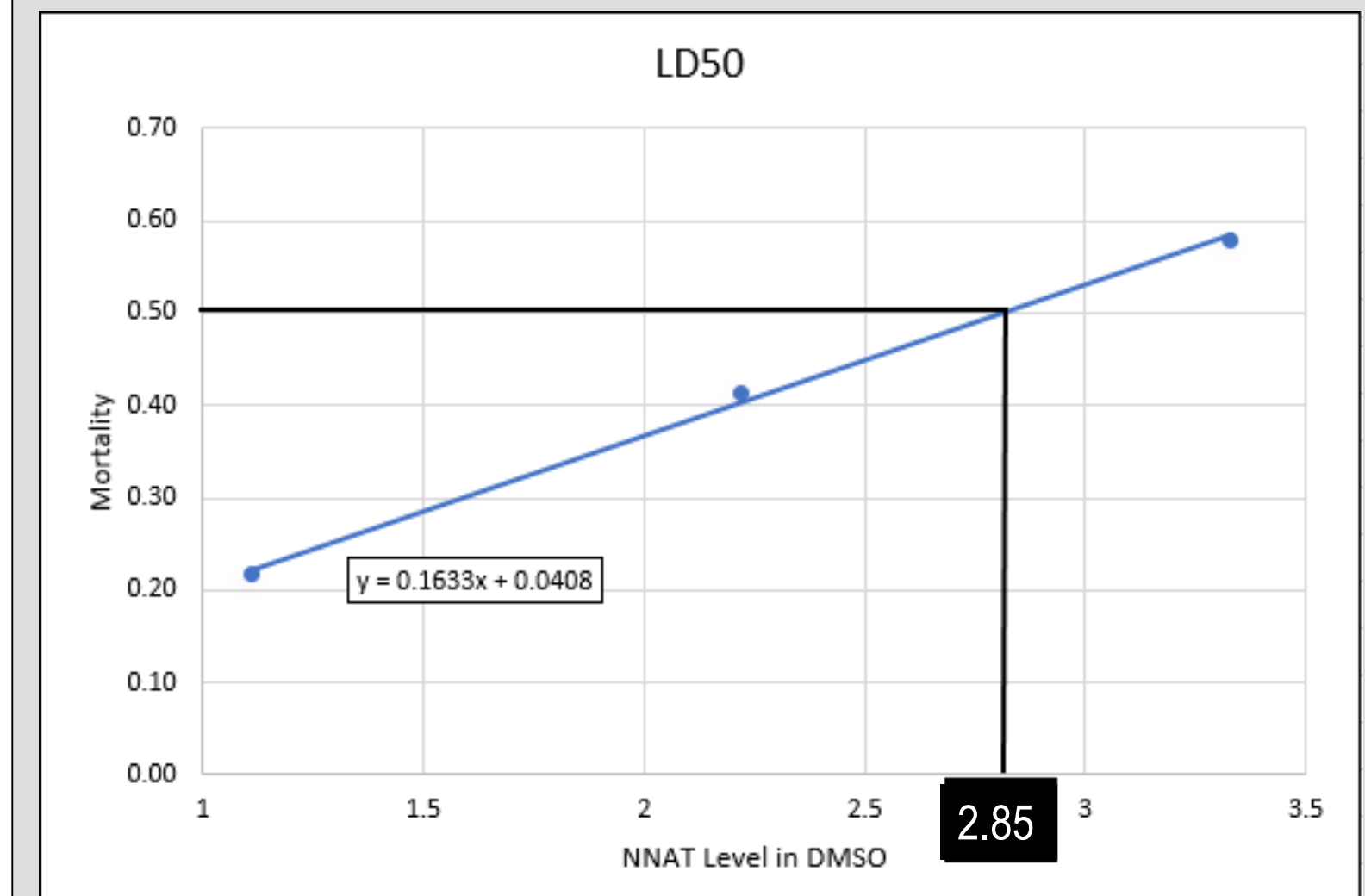
Table 5: Mean weight and Mortality Rates Experiment 2

Treatment	DMSO	50:50 Water:DMSO	NNAT 0.245 in DMSO
Mean Weight (gram)	0.1373	0.1633	0.1449
MSE	0.00355		
Mortality Probability	21.05%	0.00%*	0.00%*
Standard Error	-	-	-

Table 6: Mean weight and Mortality Rates Experiment 3

Treatment	DMSO	NNAT 1.11 in DMSO	NNAT 2.22 in DMSO	NNAT 3.33 in DMSO
Mean Weight (gram)	0.0801	0.1218	0.0972	0.0948
MSE	0.0025			
Mortality Probability	25.64 %	21.71%	41.33%	57.96%
Standard Error	0.1055	0.0898	0.1032	0.1261

LD50 plot



Based on linear regression analysis, the LD50 was determined to be 2.85 $\mu\text{mol/l}$.

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.

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- Contact Information: nfrosan@hotmail.com