Optimization of N-ethyl-N-Nitrosourea (ENU) Dose and Regime for Mutagenesis in Yoruba Nigerian Local Chicken

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INTRODUCTION

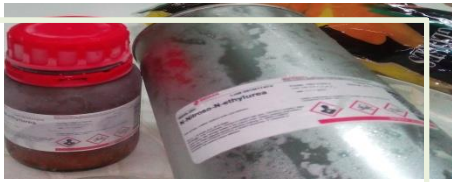
- Elucidation of the Genetic architecture of Quantitative Trait Determination is;
  - Amenable through studies of allelic series of gene mutants covering all trait determining genes.
  - A critical prerequisite for the efficient use of marker assisted selection in trait improvement and the efficient use of genotype directed animal management (pharmacogenetic, nutrigenetics. Individualized medicine) approaches in animal management during production.
- Toye *et.al.* (2004) showed that N-ethyl-N-Nitrosourea (ENU) mutagenesis can be used to determine the genetic architecture of continuously variable traits (quantitative traits)
- ENU is now routinely utilized in defining the genetic architecture of a diverse range of quantitative traits in Mice
- To date, there is no equivalent system available for use in defining the genetic architecture of Quantitative Traits in the Chicken despite the pressing need for the knowledge such a system would yield.
- To Apply ENU in defining the genetic architecture of Quantitative Traits, the safe and effective dose and regime of administration must first be defined, after which it may be deployed routinely, at the determined safe and optimal levels.

Aim

- Determination of the optimal dose and regime of ENU for mutagenesis in the chicken by use of a systematic scientific approach

Objectives

- Determination of the optimal dose and regime of ENU that produce the least percentage mortality in Y-NLC
- Determination of the optimal dose and regime of ENU that produce transient sterility and permit recovery of fertility in Y-NLC after 10 weeks post i.p injection



Materials & methods

- **Experimental Design (5 X 3 Factorial design in a CRD)**
- **Preparation of ENU and Sham (Negative Control) stocks**
- **N-Ethyl-N-Nitrosourea (ENU) dose/regime (D/R) Groups**
- **Administration of ENU**
- **Mortality Records**
- **Fertility Trial**
- **Statistical Analysis**

WORKFLOW CHART OF ENU DOSE & REGIME OPTIMIZATION IN Y-NLC

5 X 3 Factorial Experiment

ENU Dose	ENU Regime
1 0mg ENU	- one shot, ½ dose in 2 weeks & 1/3 dose in 3 weeks
2 100mg ENU	- one shot, ½ dose in 2 weeks & 1/3 dose in 3 weeks
3 200mg ENU	- one shot, ½ dose in 2 weeks & 1/3 dose in 3 weeks
4 300mg ENU	- one shot, ½ dose in 2 weeks & 1/3 dose in 3 weeks
5 400mg ENU	- one shot, ½ dose in 2 weeks & 1/3 dose in 3 weeks

0 wk

25wks

- 120 Y-NLC cocks raised from day old on standard starter and grower diets, wing tagged and randomly distributed into 15 groups of 8 cocks each at 25 weeks based on dose and regime of administration of ENU

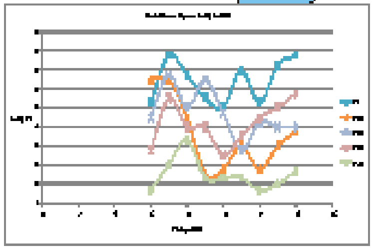
0 – 48 hrs

Mortality records post i.p. injection of ENU

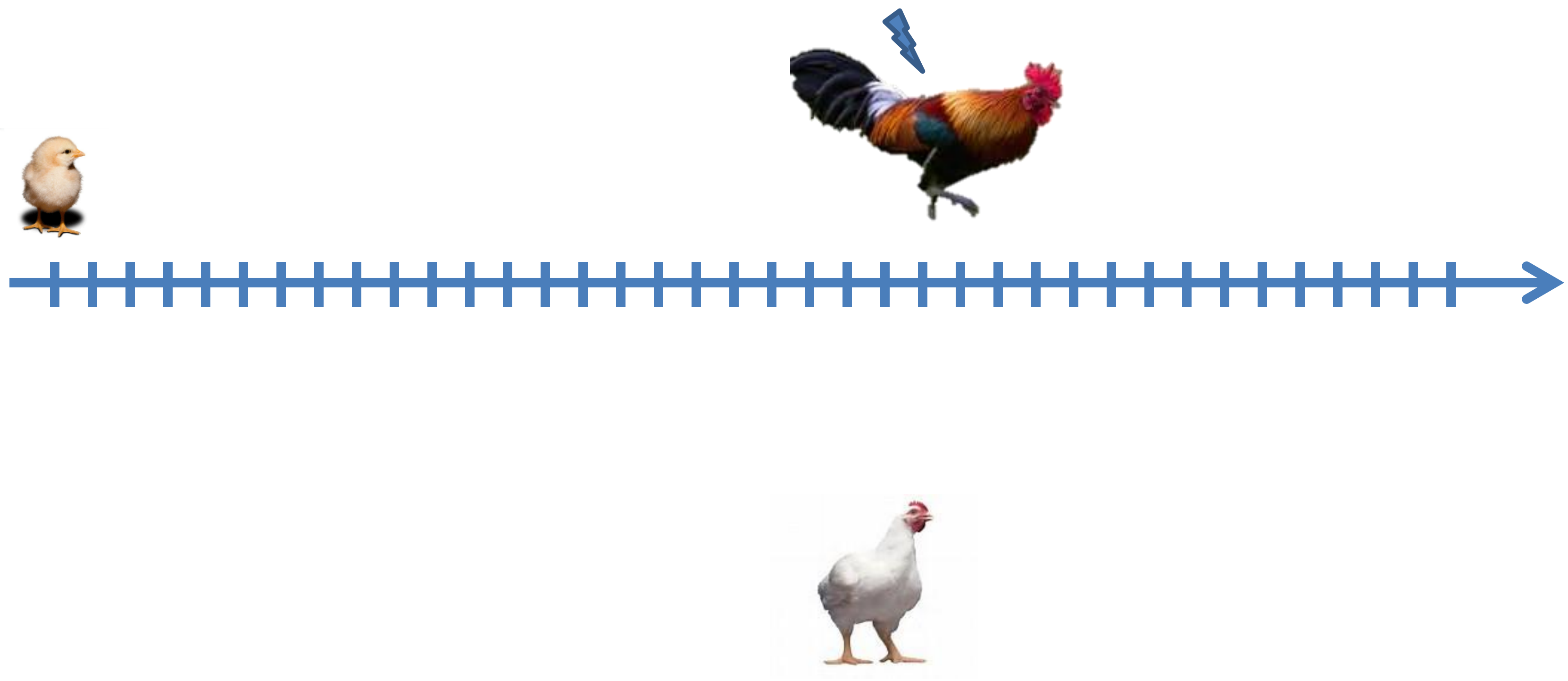
Measure of ENU Dose & Regime Lethality

5 - 14 weeks

- Each surviving Y-NLC cock was mated to 2 Issa brown hen through A.I
- Egg collected, incubated and candled on 12th day measured fertility of mutagenised Y-NLC cocks for 10wks



Analysis identified optimal ENU dose & regime that produced transient sterility and recovery of fertility after 10wks



Results & Discussions

Table 1: Table 1: Effect of Various doses and regime of Administration of N-Ethyl-N-Nitrosourea (ENU) on Fertility (±SEM) of improved Nigerian Local Chicken Cocks for 10 weeks post injection

		Weeks									
Main Effects		5	6	7	8	9	10	11	12	13	14
	Doses of ENU:										
Dose	**0 mg**	50 ± 7.52^{bc}	65 ± 8.01^{c}	60 ± 6.53^{b}	67.83 ± 5.59^{c}	60.87 ± 7.17^{bc}	61.74 ± 7.43^{c}	61.74 ± 7.54^{c}	54.78 ± 8.36^{b}	65 ± 7.96^{c}	69.57 ± 7.94^{b}
	100 mg	45.83 ± 8.21^{bc}	53.33 ± 7.86^{bc}	46.67 ± 7.86^{b}	50 ± 6.7^{bc}	38.33 ± 7.89^{b}	35 ± 7.64^{b}	45.83 ± 7.27^{bc}	33.33 ± 6.77^{ab}	41.67 ± 8.16^{bc}	53.91 ± 8.09^{b}
	200 mg	60.83 ± 7.17^{c}	58.33 ± 8.25^{c}	57.5 ± 8.19^{b}	49.17 ± 7.02^{bc}	70 ± 7.61^{c}	55 ± 7.25^{bc}	49.17 ± 8.6^{bc}	53.33 ± 8.31^{b}	40.83 ± 8.38^{bc}	54.17 ± 8.03^{b}
	300 mg	32.22 ± 8.73^{b}	32.22 ± 9.02^{b}	42.22 ± 9.55^{b}	37.78 ± 8.69^{b}	35.56 ± 8.94^{b}	33.33 ± 9.43^{b}	31.11 ± 8.28^{ab}	37.78 ± 10.4^{b}	36.67 ± 8.89^{b}	45.56 ± 9.26^{b}
	400 mg	3.08 ± 3.08^{a}	4.62 ± 3.32^{a}	15.38 ± 7.56^{a}	13.85 ± 7.3^{a}	6.15 ± 6.15^{a}	9.23 ± 6.65^{a}	12.31 ± 7.35^{a}	9.23 ± 6.25^{a}	7.69 ± 4.26^{a}	15.38 ± 8.52^{a}
	Regime of Administration:										
Regime	**Regime_1**	55.38 ± 7.71	65.38 ± 8.19	53.08 ± 7.76^{b}	46.92 ± 6.27	57.69 ± 7.39^{b}	62.31 ± 7.63^{b}	57.69 ± 7.47^{b}	40 ± 6.84	49.81 ± 7.76	59.2 ± 7.16
	Regime_2	37.89 ± 7.03	40 ± 6.75	30.81 ± 5.73^{a}	47.57 ± 6.45	44.32 ± 7.07^{ab}	37.3 ± 6.49^{a}	38.92 ± 6.43^{a}	46.49 ± 7.06	35.14 ± 6.55	48.11 ± 6.99
	Regime_3	37.89 ± 5.27	41.05 ± 5.98	58.95 ± 5.94^{b}	46.32 ± 5.6	38.95 ± 6.26^{a}	31.58 ± 5.31^{a}	36.32 ± 5.93^{a}	34.21 ± 6.53	42.11 ± 6.7	47.37 ± 6.68
	Interaction Effect:										
	Dose x Regime	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means on the same column with different superscript ^{abc} are significantly different (P< 0.05)

Table 2. Effect of Dose and Regime of N-Ethyl-N-Nitrosourea (ENU) on Mortality of improved Nigerian Local Chicken Cock

Factor	Birds (N)	Live (N)	Dead (N)	Mortality (%)
Dose (mg ENU/kg body weight)				
0	24	24	0	0^{a}
100	24	24	0	0^{a}
200	24	24	0	0^{a}
300	24	18	6	25^{b}
400	24	13	11	45.83^{c}
Regime of Administration				
1	40	27	13	32.5^{b}
2	40	38	2	5^{a}
3	40	38	2	5^{a}

Values with different superscripts ^{a,b,c} within each factor are significantly different ($p<0.05$) (Chi-squared analysis)

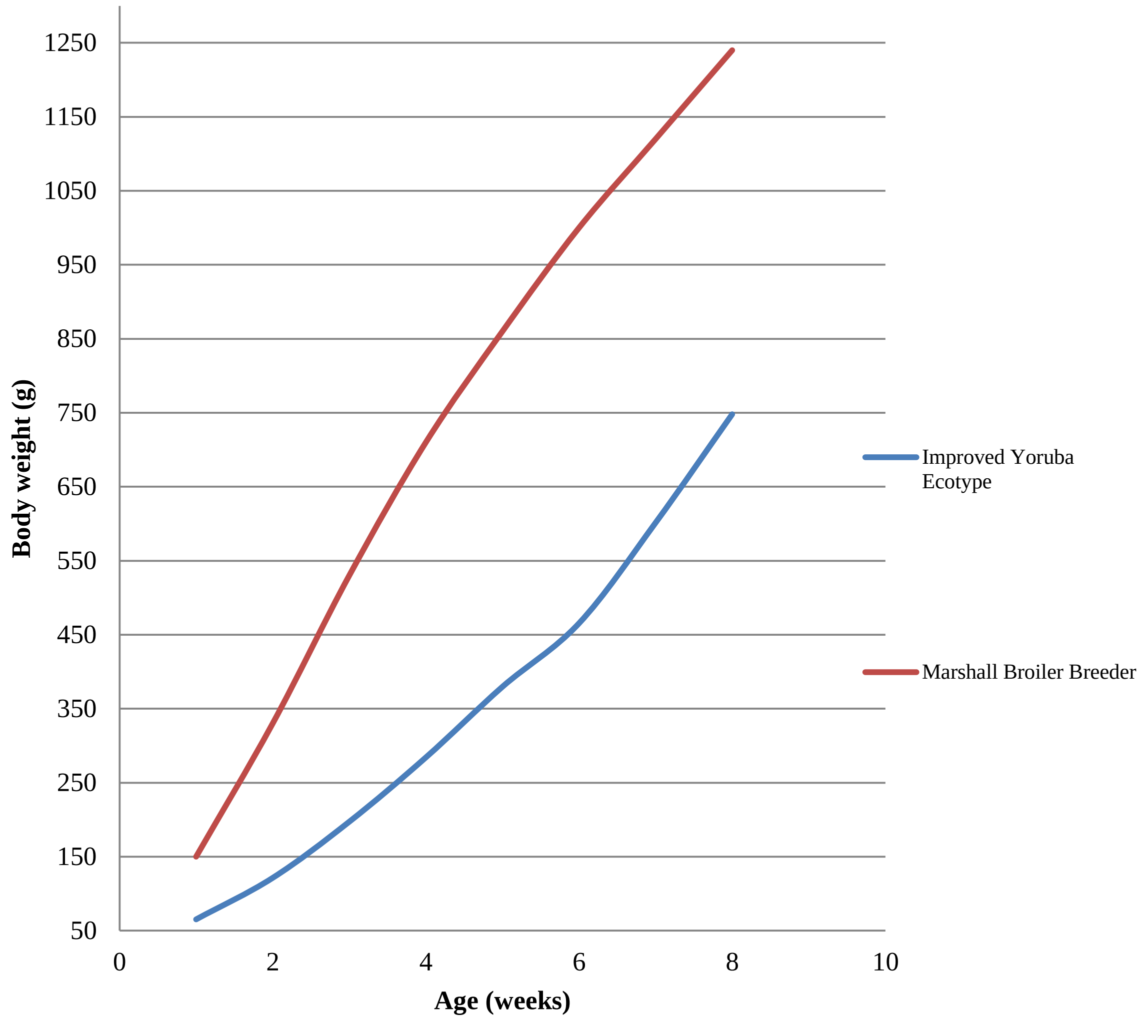


Figure 1. Body weight of Marshall broiler and Improved Yoruba Ecotype Nigerian Local Chicken from age 1 – 8

Results & Discussions

- The 400 mg/kg body weight dose of ENU resulted in significant ($p< 0.05$) loss of fertility in the cocks following ENU administration

- The 300 mg/kg body weight ENU group had a significant loss of fertility in week 6, 8, 10, 11 and 13 and regained fertility at week 14

- The 200mg/kg body weight ENU group showed no significant loss in fertility

- In the 100mg/kg body weight group, there was no significant loss of fertility except in the 10th week

Results & Discussions contd.

- In weeks 9 - 11 the groups injected with 3 weekly fractioned dose regime of ENU showed a consistent significant ($p<0.05$) loss of fertility

- Cumulative mortality within 48 hours after ENU administration was significantly determined by dose (0mg, 100 mg, 200 mg < 300 mg < 400mg) and regime (1 dose > 2 doses, 3 doses).

Conclusion

- ENU induces sterility in Y-NLC chickens
- 300 mg ENU/kg body weight administered in 3 equal fractions over 3 weeks produces transient sterility and recovery
- 400 mg ENU/kg body weight is highly lethal in Y-NLC chickens
- 300 mg ENU/kg body weight administered in 3 equal fractions is recommended for Mutagenesis in in Y-NLC chickens

Thank you for Listening

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